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DRAFT for discussion (1617)

MEMORANDUM

TO: Examiner Mojdeh Bahar

FROM: Daniel A. Monaco, Esq.

DATE: March 19, 2003

RE: U.S. Patent Application No. 09/689,281
Filed: October 11, 2000
Our File: 6056-277 (35926-149622)

Attached is a DRAFT Brief on Appeal for discussion purposes, in preparation for the interview of April 1, 2003.

DM/lma

Docket No. 06056-0127 (37525/149522) PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent application of: Stephen C. Cosenza, *et al.*

Application No.: 09/689,281

Group Art Unit: 1617

Examiner: Bahar, M.

Filed: October 11, 2000

For: METHOD FOR PROTECTING NORMAL CELLS FROM CYTOTOXICITY OF CHEMOTHERAPEUTIC AGENTS

DRAFT

BRIEF ON APPEAL

FOR INTERVIEW ONLY; NOT FOR ENTRY.

BOX PAI
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Pursuant to the provisions of 37 C.F.R. § 1.191 and § 1.192, an appeal is taken herein from the rejection dated October 11, 2003, which rejects claims 1-7 and 12-22 of this application. Appellants submit herewith the following attachments: Attachment (1), an original and two copies of this Appeal Brief; and Attachment (2), three copies of each exhibit cited in the Appeal Brief, including: (a) Exhibit A, a copy of the claims on appeal; (b) Exhibit B, a copy of Reddy, *et al.*, WO 99/18608 published on April 15, 1999; (c) Exhibit C, a copy Griggs, Embase Abstract AN 1998287056, copyright 2001; (d) Exhibit D, an entry for amifostine from The Merck Index 12th Edit., Merck & Co., Inc., Whitehouse Station, NJ (1996); and (e) Exhibit E, a copy of an abstract entitled "Cytoprotectant Amifostine Approved".

REAL PARTY IN INTEREST

The real party of interest is the assignee of the above-identified application: Temple University Of The Commonwealth System Of Higher Education.

RELATED APPEALS AND INTERFERENCES

Appellants and their legal representatives hereby submit that they are not aware of any appeal or interference which directly affects, will be directly affected by, or will have a bearing on the Board's decision in this appeal.

STATUS OF THE CLAIMS

Claims 1-7 and 12-22 of this application have been twice rejected and are the subject of this appeal. Claims 8-11 were previously canceled without prejudice. Appellants timely filed a Notice of Appeal to the Board of Patent Appeals and Interferences on _____. The appealed claims are presented in Exhibit A attached hereto.

SUMMARY OF THE INVENTION

The invention as recited by the claims on appeal encompasses a method of protecting animals, inclusive of humans, from cytotoxic side effects of anticancer drugs, namely, mitotic phase cell cycle or topoisomerase inhibitors, which comprises administering at least one cytoprotective α, β unsaturated aryl sulfone compound as a cytoprotective agent prior to treatment with mitotic phase cell cycle or topoisomerase inhibitors. The inventors of the present

invention discovered for the first time that α, β unsaturated aryl sulfones can be used as cytoprotective agents prior to the administration of an anticancer drug to prevent or reduce cytotoxicity resulting from the administration of the anticancer drug. At the time the invention was made, α, β unsaturated aryl sulfones were known to exhibit specific cytotoxicity towards tumor cells. However, the use of α, β unsaturated aryl sulfones as a cytoprotective agent prior to the administration of an anticancer drug has never been reported or known before the present invention was made.

ISSUE ON APPEAL

The sole issue presented by this appeal is: whether the rejection under 35 U.S.C. § 103(a) of claims 1-7 and 12-22 over Reddy *et al.* WO 99/18608 (herein after, "Reddy") in view of Griggs, Embase Abstract AN 1998287056 (hereinafter, "Griggs") can be maintained despite the lack of the legally required teaching and/or suggestion of the invention by the cited references, and despite the lack of legally required motivation to combine the cited references?

GROUPING OF CLAIMS

Claims 1-7 and 12-22 stand or fall together.

REFERENCES RELIED UPON BY THE EXAMINER

Primary: Reddy, submitted herewith as Exhibit B, which was published on April 15, 1999, discloses certain styryl sulfones as specific anticancer agents. This publication does not

teach or suggest the use of α, β unsaturated aryl sulfones as protective agents against cytotoxic effects of mitotic phase cell cycle or topoisomerase inhibitors.

Secondary: Griggs, submitted herewith as Exhibit C, which was published in 1998, merely states that amifostine is a cytoprotective agent that reduces treatment-related toxicity of certain anticancer therapies namely alkylating, platinum, paclitaxel and radiation therapies. Griggs not only fails to teach the use of α, β unsaturated aryl sulfones as protective agents in cancer therapy but teaches the use of a cytoprotective compound whose structure, function and mechanism of action are completely different from the α, β unsaturated aryl sulfone compounds as claimed.

ARGUMENT

Appellants respectfully request that the Board of Patent Appeals and Interferences ("the Board") reverse the Examiner's rejection of the pending claims. As discussed below, the Examiner has erred not only in improperly combining the Reddy and Griggs references but also in failing to appreciate that the appealed claims can be distinguished over that combination.

I. THE LEGAL REQUIREMENTS FOR ESTABLISHING A *PRIMA FACIE* CASE OF OBVIOUSNESS

As the Board is well aware, three basic criteria must be met to establish a case of *prima facie* obviousness under 35 U.S.C. § 103.

First, there must have been at the time of the invention a motivation to combine the references cited. *In re Jones*, 958 F.2d 347 (Fed. Cir. 1992); *In re Fine*, 837 F.2d 1071, 1075

(Fed. Cir. 1988)(holding that obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art). *Ecolochem, Inc., v. Southern California Edison Company*, 227 F.3d 1361, 1372 (Fed. Cir. 2000), citing *ACS Hosp. Sys., Inc. v. Montefiore Hosp.*, 732 F.2d 1572, 1577 (Fed. Cir. 1984)(holding obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination).

Second, the alleged prior art must teach or suggest all of the limitations of the claims alleged to be obvious. *In re Royka,* 490 F.2d 981 (CCPA 1974)(holding that to establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art).

Third, there must have been at the time of the invention a reasonable expectation of success for arriving at the claimed invention. *In re Vaeck,* 947 F.2d 488 (Fed. Cir. 1991)(holding that the teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in the applicant's disclosure); *Amgen, Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 1207-1208 (Fed. Cir.), cert. denied 502 U.S. 856 (1991) *In re Deuel,* 51 F.3d 1552, 1558 (Fed. Cir. 1995).

B. THE USE OF HINDSIGHT IS IMPERMISSIBLE

Hindsight cannot be used to reject a claim as obvious. *In re Sernaker*, 702 F.2d 989, 994 (Fed. Cir. 1983); *In re Rinehart*, 531 F.2d 1048 (CCPA 1976); *In re Imperato*, 486 F.2d 585 (CCPA 1973); *In re Adams,* 356 F.2d 998 (CCPA 1966). Consequently, it is legally improper to select from the prior art the separate components of the inventor's combination, using the blueprint supplied by the inventor. *C.R. Bard Inc. v. M3 Systems, Inc.*, 157 F.3d 1340, 1352 (Fed. Cir. 1998) citing *Fromson v. Advance Offset Plate, Inc.*, 755 F.2d 1549, 1556 (Fed. Cir. 1985)(holding the prior art must suggest to one of ordinary skill in the art the desirability of the claimed combination).

The Federal Circuit has suggested that "the best defense against the subtle but powerful attraction of a hindsight-based obviousness analysis is rigorous application of the requirement for a showing of the teaching or motivation to combine prior art references. *Id.* This is because "when prior art references require selective combination by the court to render obvious a subsequent invention, there must be some reason for the combination other than the hindsight gleaned from the invention itself." *Interconnect Planning Corp. v. Feil*, 774 F.2d 1132, 1142 (Fed. Cir. 1985).

II. THE PENDING CLAIMS ARE NOT OBVIOUS OVER THE CITED REFERENCES

The Examiner rejected Claims 1-7 and 12-22 under 35 U.S.C. § 103 as allegedly being obvious over Reddy in view of Griggs. The Examiner asserts that Reddy teaches styryl sulfone compounds employed in a method of treating breast and prostate tumor cells, and induce

apoptosis of such tumor cells while sparing normal cells. Specifically, the Examiner states that:

> [R]eddy teaches that its aryl sulfones are effective anti-tumor agents, yet they "spare" normal cells. The sparing of the normal cells means that the incidence of side effects (on normal cells) is reduced if not eliminated. Therefore Reddy does indeed teach the employment of aryl sulfones as cytoprotective agents.

The Office Action, Paper No. 16, page 4, lines 5-9.

While the Examiner acknowledges that Reddy does not teach a method of administration of an α, β unsaturated aryl sulfone prior to the administration of mitotic phase cell cycle or topoisomerase inhibitors, it is the Examiner's position that Griggs allegedly cures this deficiency by teaching that cytoprotective agents used in combination with anticancer therapy reduce treatment-related toxicity of anticancer therapy. The Examiner, therefore, alleges that it would have been obvious to one of ordinary skill in the art at the time the invention was made to employ an anticancer drug along with a cytoprotective agent such as the styryl sulfone compounds of Reddy to arrive at the claimed invention. To provide the requisite motivation to combine the references, the Examiner specifically states that:

> [o]ne of ordinary skill in the art would have been motivated to employ an anti-cancer drug along with a cytoprotective agent such as the styryl sulfone compound of Reddy *et al.* because both compounds are known to be effective in treating cancer and that it is *prima facie* obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be useful for the very same purpose.

Office Action, Paper No. 16, page 3, lines 10-14.

A. THE CITED REFERENCES DO NOT SUGGEST THE CLAIMED INVENTION

Claims 1-7 and 12-22 are directed to methods in which an animal (inclusive of humans) is protected from cytotoxic side effects of mitotic phase cell cycle or topoisomerase inhibitors by administering at least one cytoprotective α, β unsaturated aryl sulfone compound before treatment with the mitotic phase cell cycle or topoisomerase inhibitors.

Appellants respectfully submit that the Examiner has failed to make a *prima facie* case of obviousness against the invention claimed. The Examiner has the burden under 35 U.S.C. § 103 to establish a *prima facie* case of obviousness. *In re Piasecki*, 745 F.2d 1468, 1471-1472 (Fed. Cir. 1984). The Examiner can satisfy this burden only by demonstrating some objective teaching in the prior art or showing that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references. *In re Lalu*, 747 F.2d 703, 705 (Fed. Cir. 1984). Additionally, the Examiner must show that the references properly combined teach each and every element of the claimed invention. Appellants respectfully submit that the Examiner has not met this burden.

Neither Reddy nor Griggs, either alone or in combination, teaches or suggests the invention as claimed. The legally required suggestion of each and every element of the pending claims, namely, a specific method of therapy for "protection of an animal", from a specific disorder, "cytotoxic side effects" resulting from the use of a specific class of anticancer compounds, "mitotic phase cell cycle or topoisomerase inhibitors", by the use of a specific compound, "α, β unsaturated aryl sulfone", in a specific manner, "prior to the administration of

the mitotic phase cell cycle or topoisomerase inhibitors", are simply not present in Reddy or Griggs, either alone or in combination.

Specifically, there is no suggestion in Reddy for the use of an α, β unsaturated aryl sulfone as a cytoprotective agent. The Examiner alleges that Reddy teaches that aryl sulfone compounds are cytoprotective agents because Reddy states that these compounds are cytotoxic to tumor cells but "spare normal cells." Appellants respectfully submit that the Examiner's characterization of this statement is incorrect. Contrary to the Examiner's assertion, the statement in Reddy, regarding sparing of normal cells, does not teach the use of aryl sulfone compounds as a cytoprotective agent. Rather, the statement merely suggests that aryl sulfone compounds are specific anticancer drugs that exclusively attack cancer cells, while not harming normal cells.

Appellants respectfully submit that the specificity of an anticancer drug against a particular cancer does not render that anticancer drug a "cytoprotective agent" for normal cells. If the interpretation of the Examiner for the phrase "sparing normal cells" was correct, then a majority of the current anticancer drugs that specifically affect cancer cells while sparing normal cells would have also been regarded as cytoprotective agents. Clearly, this is not the case.

The Examiner further alleges that

> "[A]ssuming *arguendo* that Reddy does not teach the cytoprotective qualities of aryl sulfones, at the very least Reddy teaches that styryl sulfone compounds are known to be effective in treating cancer. . . It is *prima facie* obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be useful for the very same purpose."

The Examiner appears to suggest that it would be obvious to combine Reddy and Griggs as each separately teaching an anticancer agent. The Examiner's position is untenable for the following reasons.

First, contrary to the Examiner's contention, Griggs does not teach that amifestone is an anticancer drug. Second, assuming, *arguendo,* that it was obvious to combine Griggs and Reddy, on the basis of the Examiner's incorrect interpretation that Griggs teaches an anticancer agent, there can be no teaching or suggestion in the combination for a method of protecting normal cells from cytotoxicity as claimed. Third, the combined references do not teach the order of administration of the first and second drug. The appealed claims specifically require administration of an α, β unsaturated aryl sulfone compound prior to the administration of mitotic phase cell cycle or topoisomerase inhibitors. The specification specifically teaches that simultaneous exposure of α, β unsaturated aryl sulfone and the inhibitor does not result in protection. *See,* specification at page 15, last paragraph.

Griggs merely discloses that amifostine protects normal cells from the cytotoxic effects of alkylating agents, paclitaxel, and radiation. Amifostine is an organic thiophosphate homolog of cytsteamine; *see* entry for "amifostine" from The Merck Index (12th Edit.), Budavari *et al., eds.*, Merck & Co., Inc., Whitehouse Station, NJ, 1996, attached herewith as Exhibit D. It was also known at the time the present application was filed that more amifostine is taken up by normal cells than by tumor cells, which accounts for the drug's cytoprotective properties. *See, e.g.*, the abstract entitled "Cytoprotectant Amifostine Approved" accessed from

http://www.slip.net/~mcdavis/database/amifos2.htm

on July 18, 2002, attached herewith as Exhibit E, which references Spencer, C.M., *et al.*, (1995), *Drugs* 50: 1001-1031.

There is no mention in Griggs of α, β unsaturated aryl sulfone compounds, less so their use as a cytoprotective agent equivalent to amifostine. Even assuming *arguendo* that Reddy somehow teaches the use of α, β unsaturated aryl sulfones as cytoprotectants, the mere fact that both compounds are cytoprotectants is not sufficient, absent some other indication in the prior art, that amifostine and α, β unsaturated aryl sulfones are interchangeable. M.P.E.P. 2144.06. The Examiner has not identified any teaching in the prior art showing that amifostine and the α, β unsaturated aryl sulfones are equivalent cytoprotectants.

As can be seen from the Merck Index entry, *supra*, the chemical structures of the α, β unsaturated aryl sulfones and amifostine are completely unrelated. There is also no evidence in Reddy or Griggs that α, β unsaturated aryl sulfones have a differential uptake in normal *vs.* tumor cells akin to amifostine. Moreover, the mechanism of action of amifostine and α, β unsaturated aryl sulfones appears to be different: amifostine protects normal cells from alkylating agents (*see*, Griggs), while α, β unsaturated aryl sulfones do not (*see*, the specification at page 51, lines 16-19 and Table 6).

Amifostine and α, β unsaturated aryl sulfones disparate chemical structures, different mechanisms of action, and potentially dissimilar pharmacology demonstrate that these compounds are not equivalent. Thus, even if the Examiner's interpretation of Reddy were taken as correct, one of ordinary skill in the art following the teachings of Reddy and Griggs would not arrive at the presently claimed invention.

As the Board has asserted in *Ex parte Clapp*, "[t]o support the conclusion that the claimed invention is directed to obvious subject matter, either the references must expressly or impliedly suggest the claimed invention or the Examiner must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references." *Ex parte Clapp*, 227 USPQ 972, 973 (Bd. Pat. App. & Inter. 1985). In this case, the cited references do not suggest the claimed invention because 1) Reddy does not disclose or suggest that α, β unsaturated aryl sulfone compounds are cytoprotective agents; 2) Reddy does not disclose or suggest the administration of α, β unsaturated aryl sulfone compounds prior to administering a separate mitotic phase cell cycle or topoisomerase inhibitor for protecting normal cells from those inhibitors; 3) the chemical structure of the α, β unsaturated aryl sulfones and amifostine are unrelated; and 4) the Reddy's α, β unsaturated aryl sulfones and amifostine do not share similar pharmacokinetic characteristics.

Accordingly, Appellants submit that the cited references, either alone or in combination, does not teach or suggest each and every element of the claimed invention. Therefore, the Examiner's rejection must be reversed.

B. NO MOTIVATION TO COMBINE THE REFRENCES

Appellants respectfully submit that the Examiner has improperly combined the references. The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F.2d 680, 682 (Fed. Cir. 1990)(emphasis added) citing *In re Gordon*, 733 F.2d 900, 902 (Fed. Cir. 1984)(the mere fact that the prior art could be so modified

would not have made the modification obvious unless the prior art suggested the desirability of the modification). In the instant case, there is no such suggestion to combine the references cited by the Examiner.

One of ordinary skill in the art following the teachings of Reddy would have no need, desire, or reason to look to a reference that suggests the use of a cytoprotective agent to protect normal cells from the adverse effects of an anticancer drug simply because Reddy itself teaches that aryl sulfone compounds are anticancer drugs that do not harm normal cells. Similarly, one of ordinary skill in the art following the teachings of Griggs would have no need, desire, or reason to look to Reddy for the use of aryl sulfones, either as an anticancer drug or as a cytoprotective agent. Grigg's method achieves cytoprotection by using the specific compound amifestone which is not an aryl sulfone. Griggs' anticancer regimen does not include the use of an aryl sulfone compound as an anticancer drug and its cytoprotective agent "amifestone" is very different from aryl sulfones. Neither Reddy nor Griggs suggests the desirability of using a specific anticancer drug as a cytoprotective agent prior to the administration of a second anticancer drug, as required by the appealed claims. Further, Griggs contains no teaching of the timing of administration of amifestone. Thus, one of ordinary skill in the art would have no motivation to combine the teachings of Reddy and Griggs to arrive at the present invention as claimed.

Because there is no teaching or suggestion for using α, β unsaturated aryl sulfone compounds as cytoprotectants before an anticancer drug prior to Applicants' disclosure, the Examiner's interpretation of Reddy and Griggs must derive from impermissible hindsight reconstruction using the present specification. As the Federal Circuit held in *In re Gorman*, 18

USPQ2d 1885, 1888 (Fed. Cir. 1991), it is improper to combine prior art teachings to render the claimed invention obvious, absent some "teaching, suggestion or incentive supporting the combination." *See, also, Uniroyal Inc. v. Rudkin-Wiley Corp.* 5 USPQ2d 1434 (Fed. Cir. 1988)("When prior art references require selective combination by the court to render obvious a subsequent invention, there must be some reason for the combination other than the hindsight gleaned from the invention itself") and *In re Rouffet*, 47 USPQ2d 1453, 1456 (Fed. Cir. 1998). Thus, the prior art documents as a whole must provide the motivation for making the combination, and this motivation must be found apart from Applicant's disclosure. *In re Dow Chemical Co.*, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988). Absent such motivation, however, the Examiner may not use the claimed invention as a template to piece together elements from various unrelated prior art documents, or use an isolated teaching from a reference to fill gaps in the prior art, to arrive at an obviousness rejection. *In re Gorman, supra*, at 1888; *In re Fritch,* 23 USPQ2d 1780, 1784 (Fed. Cir. 1992).

Here, the cited references carry no relationship except that imposed by the present specification and claims. Once removed from the context of Applicant's disclosure, the references fragment into a collection of unrelated disclosures with little bearing on the claimed invention. Any motivation to combine the teachings of the cited references is the result of impermissible hindsight. Thus, Appellants submit that the requisite suggestion or motivation for one of ordinary skill in the art to arrive at the elements of the claimed invention is absent. Because the suggestion to combine Reddy and Griggs is absent from the cited references, the Board should overturn the Examiner's rejection and allow the pending claims.

CONCLUSION

Neither Reddy nor Griggs taken alone or in combination suggests a method of protecting humans from cytotoxic side effects of anticancer drugs, namely, mitotic phase cell cycle or topoisomerase inhibitors, by the use of the specific compound of α, β unsaturated aryl sulfone prior to treatment with mitotic phase cell cycle or topoisomerase inhibitors. Additionally, there is no motivation to combine the cited references other than the hindsight gleaned from the invention itself.

Respectfully submitted,

STEPHEN C. COSENZA *et al.*

DRAFT

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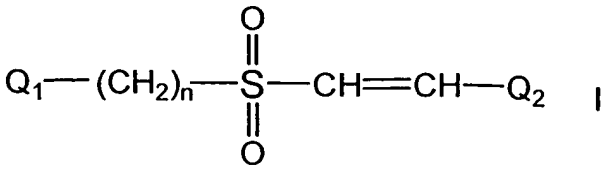
EXHIBIT A

EXHIBIT A

Claims on appeal

1. A method for protecting an animal from cytotoxic side effects of the administration of a mitotic phase cell cycle inhibitor or a topoisomerase inhibitor comprising administering to the animal, in advance of administration of said inhibitor, an effective amount of at least one cytoprotective α, β unsaturated aryl sulfone compound, wherein the mitotic phase cell cycle inhibitor and topoisomerase inhibitor are other than an α, β unsaturated aryl sulfone compound.

2. A method according to claim 1 wherein the cytoprotective compound has the formula I:



I

wherein:

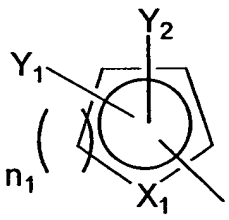
n is one or zero;

Q_1 and Q_2 are, same or different, are substituted or unsubstituted aryl; or

a pharmaceutically acceptable salt thereof.

3. The method according to claim 2 wherein:

Q_1 is selected from the group consisting of substituted and unsubstituted phenyl, 1-naphthyl, 2-naphthyl, 9-anthryl and an aromatic radical of formula II:



II

wherein

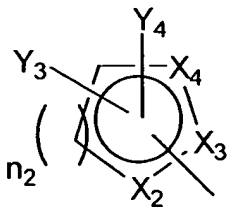
n_1 is 1 or 2,

Y_1 and Y_2 are independently selected from the group consisting of hydrogen, halogen, and nitro, and

X_1 is selected from the group consisting of oxygen, nitrogen, sulfur and

; and

Q_2 is selected from the group consisting of substituted and unsubstituted phenyl, 1-naphthyl, 2-naphthyl, 9-anthryl and an aromatic radical of formula III:



III

wherein

n_2 is 1 or 2,

Y_3 and Y_4 are independently selected from the group consisting of hydrogen, halogen, and nitro, and

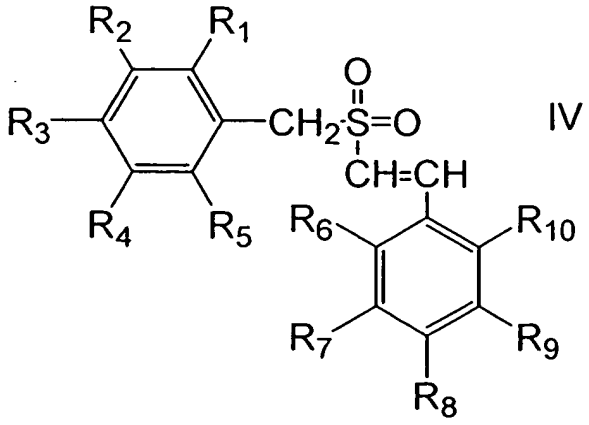
X_2, X_3 and X_4 are independently selected from the group consisting of carbon, oxygen, nitrogen, sulfur and

provided that not all of X_2, X_3 and X_4 may be carbon; or

a pharmaceutically acceptable salt thereof.

4. (Once Amended) A method according to claim 3 wherein Q_1 and Q_2 are selected from substituted and unsubstituted phenyl.

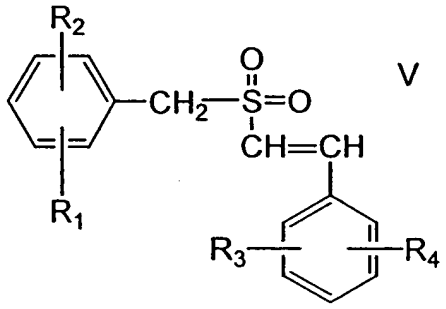
5. A method according to claim 4 wherein the cytoprotective compound has the formula IV:



wherein:

R_1 through R_{10} are independently selected from the group consisting of hydrogen, halogen, C1-C8 alkyl, C1-C8 alkoxy, nitro, cyano, carboxy, hydroxy, phosphonato, amino, sulfamyl, acetoxy, dimethylamino(C2-C6 alkoxy), C1-C6 trifluoroalkoxy and trifluoromethyl; or a pharmaceutically acceptable salt thereof.

6. The method according to claim 4 wherein the cytoprotective compound has the formula V:



V

wherein R_1, R_2, R_3 and R_4 are independently selected from the group consisting of hydrogen, halogen, C1-C8 alkyl, C1-C8 alkoxy, nitro, cyano, carboxy, hydroxy and trifluoromethyl; or a pharmaceutically acceptable salt thereof.

7. The method of claim 6 wherein the cytoprotective compound is selected from the group consisting of (E)-4-fluorostyryl-4-chlorobenzylsulfone; (E)-2-chloro-4-fluorostyryl-4-

chlorobenzylsulfone; (E)-4-chlorostyryl-4-chlorobenzylsulfone; (E)-4-carboxystyryl-4-chlorobenzyl sulfone; and (E)-4-fluorostyryl-2,4-dichlorobenzylsulfone.

12. The method of claim 1 wherein the cytoprotective compound is of the Z-configuration.

13. The method according to claim 1 wherein the cytoprotective compound is administered at least about 4 hours before administration of the mitotic phase cell cycle inhibitor or topoisomerase inhibitor.

14. The method according to claim 13 wherein the cytoprotective compound is administered at least about 12 hours before administration of the mitotic phase cell cycle inhibitor or topoisomerase inhibitor.

15. The method according to claim 14 wherein the cytoprotective compound is administered at least about 24 hours before administration of the mitotic phase cell cycle inhibitor or topoisomerase inhibitor.

16. The method according to claim 13 wherein the mitotic phase cell cycle inhibitor is selected from the group consisting of vinca alkaloids, taxanes, naturally occurring macrolides,

and colchicine and its derivatives; and the topoisomerase inhibitor is selected from the group consisting of camptothecin, etoposide and mitoxantrone.

17. The method according to claim 16 wherein the mitotic phase cell cycle inhibitor is selected from the group consisting of paclitaxel and vincristine.

18. In a method for treating cancer or other proliferative disorder comprising administering an effective amount of at least one mitotic phase cell cycle inhibitor or topoisomerase inhibitor to an animal in need of such treatment, the improvement comprising administering to said animal prior to administration of said mitotic phase cell cycle inhibitor or topoisomerase inhibitor an effective amount at least one cytoprotective α, β unsaturated aryl sulfone compound, wherein the mitotic phase cell cycle inhibitor and topoisomerase inhibitor are other than an α, β unsaturated aryl sulfone compound, and wherein the animal is protected from the cytotoxic side effects of the administration of said mitotic phase cell cycle inhibitor or topoisomerase inhibitor.

19. The method according to claim 18 wherein the cytoprotective compound is administered at least about 4 hours before administration of the mitotic phase cell cycle inhibitor or topoisomerase inhibitor.

20. The method according to claim 19 wherein the cytoprotective compound is administered at least about 12 hours before administration of the mitotic phase cell cycle inhibitor or topoisomerase inhibitor.

21. The method according to claim 20 wherein the cytoprotective compound is administered at least about 24 hours before administration of the mitotic phase cell cycle inhibitor or topoisomerase inhibitor.

22. The method of claim 18 wherein the cytoprotective compound is selected from the group consisting of (E)-4-fluorostyryl-4-chlorobenzylsulfone; (E)-2-chloro-4-fluorostyryl-4-chlorobenzylsulfone; (E)-4-chlorostyryl-4-chlorobenzylsulfone; (E)-4-carboxystyryl-4-chlorobenzyl sulfone; and (E)-4-fluorostyryl-2,4-dichlorobenzylsulfone.

EXHIBIT B

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07C 317/02, 317/14

A1

(11) International Publication Number: WO 99/18068

(43) International Publication Date: 15 April 1999 (15.04.99)

(21) International Application Number: PCT/US98/20580

(22) International Filing Date: 1 October 1998 (01.10.98)

(30) Priority Data:
60/060,933 3 October 1997 (03.10.97) US

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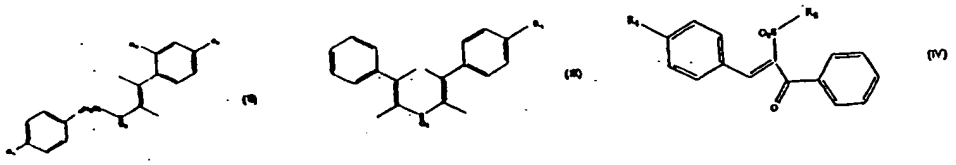
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, US, UZ, VN, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
With amended claims.

(54) Title: STYRYL SULFONE ANTICANCER AGENTS



(57) Abstract

Styryl sulfone compounds of the invention selectively inhibit proliferation of breast and prostate tumor cells, and induce apoptosis of such tumor cells, while sparing normal cells. The compounds, which are useful in the treatment of breast or prostate cancer, have formula (II) wherein n is zero or one; R_1 is selected from the group consisting of hydrogen, chlorine, fluorine and bromine; R_2 is selected from the group consisting of hydrogen, chlorine, fluorine, bromine, methyl and methoxy; and R_3 is selected from the group consisting of hydrogen, chlorine and fluorine; provided, R_2 may not be methyl or methoxy when R_1 and R_3 are both hydrogen and n is zero or one; and R_1, R_2 and R_3 may not all be hydrogen when n is one, or formula (III) wherein R_1 is selected from the group consisting of hydrogen, chlorine, fluorine and bromine or formula (IV) wherein R_1 is selected from the group consisting of fluorine and bromine, and R_2 is selected from the group consisting of 2-chlorophenyl, 4-chlorophenyl, 4-fluorophenyl and 4-nitro.

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STYRYL SULFONE ANTICANCER AGENTS

Field of the Invention

The invention relates to compositions and methods for the treatment of cancer, in particular breast and prostate cancer.

Background of the Invention

Extracellular signals received at transmembrane receptors are relayed into the cells by the signal transduction pathways (Pelech *et al.*, *Science* 257:1335 (1992)) which have been implicated in a wide array of physiological processes such as induction of cell proliferation, differentiation or apoptosis (Davis *et al.*, *J. Biol. Chem.* 268:14553 (1993)). The Mitogen Activated Protein Kinase (MAPK) cascade is a major signaling system by which cells transduce extracellular cues into intracellular responses (Nishida *et al.*, *Trends Biochem. Sci.* 18:128 (1993); Blumer *et al.*, *Trends Biochem. Sci.* 19:236 (1994)). Many steps of this cascade are conserved, and homologous for MAP kinases have been discovered in different species.

In mammalian cells, the Extracellular-Signal-Regulated Kinases (ERKs), ERK-1 and ERK-2 are the archetypal and best-studied members of the MAPK family, which all have the unique feature of being activated by phosphorylation on threonine and tyrosine residues by an upstream dual specificity kinase (Posada *et al.*, *Science* 255:212 (1992); Biggs III *et al.*, *Proc. Natl. Acad. Sci. USA* 89:6295 (1992); Garner *et al.*, *Genes Dev.* 6:1280 (1992)).

Recent studies have identified an additional subgroup of MAPKs, known as c-Jun NH2-terminal kinases 1 and 2 (JNK-1 and JNK-2), that have different substrate specificities and are regulated by different stimuli (Hibi *et al.*, *Genes Dev.* 7:2135 (1993)). JNKs are members of the class of stress-activated

protein kinases (SPKs). JNKs have been shown to be activated by treatment of cells with UV radiation, pro-inflammatory cytokines and environmental stress (Derijard *et al.*, *Cell* 1025 (1994)). The activated JNK binds to the amino terminus of the c-Jun protein and increases the protein's transcriptional activity by phosphorylating it at ser63 and ser73 (Adler *et al.*, *Proc. Natl. Acad. Sci. USA* 89:5341 (1992); Kwok *et al.*, *Nature* 370:223 (1994)).

Analysis of the deduced primary sequence of the JNKs indicates that they are distantly related to ERKs (Davis, *Trends Biochem. Sci.* 19:470 (1994)). Both ERKs and JNKs are phosphorylated on Tyr and Thr in response to external stimuli resulting in their activation (Davis, *Trends Biochem. Sci.* 19:470 (1994)). The phosphorylation (Thr and Tyr) sites, which play a critical role in their activation are conserved between ERKs and JNKs (Davis, *Trends Biochem. Sci.* 19:470 (1994)). However, these sites of phosphorylation are located within distinct dual phosphorylation motifs: Thr-Pro-Tyr (JNK) and Thr-Glu-Tyr (ERK). Phosphorylation of MAPKs and JNKs by an external signal often involves the activation of protein tyrosine kinases (PTKs) (Gille *et al.*, *Nature* 358:414 (1992)), which constitute a large family of proteins encompassing several growth factor receptors and other signal transducing molecules.

Protein tyrosine kinases are enzymes which catalyze a well defined chemical reaction: the phosphorylation of a tyrosine residue (Hunter *et al.*, *Annu Rev Biochem* 54:897 (1985)). Receptor tyrosine kinases in particular are attractive targets for drug design since blockers for the substrate domain of these kinases is likely to yield an effective and selective antiproliferative agent. The potential use of protein tyrosine kinase blockers as antiproliferative agents was recognized as early as 1981, when quercetin was suggested as a PTK blocker (Graziani *et al.*, *Eur. J. Biochem.* 135:583-589 (1983)).

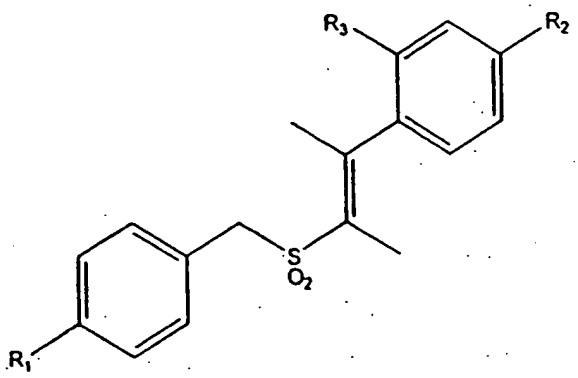
The best understood MAPK pathway involves extracellular signal-regulated kinases which constitute the Ras/Raf/MEK/ERK kinase cascade (Boudewijn *et al.*, *Trends Biochem. Sci.* 20, 18 (1995)). Once this pathway is activated by different stimuli, MAPK phosphorylates a variety of proteins including several transcription factors which translocate into the nucleus and activate gene

transcription. Negative regulation of this pathway could arrest the cascade of these events.

What are needed are new anticancer chemotherapeutic agents which target receptor tyrosine kinases and which arrest the Ras/Raf/MEK/ERK kinase cascade. Oncoproteins in general, and signal transducing proteins in particular, are likely to be more selective targets for chemotherapy because they represent a subclass of proteins whose activities are essential for cell proliferation, and because their activities are greatly amplified in proliferative diseases.

Summary of the Invention

According to one embodiment of the invention, novel compounds are provided according to formula I:



I

wherein

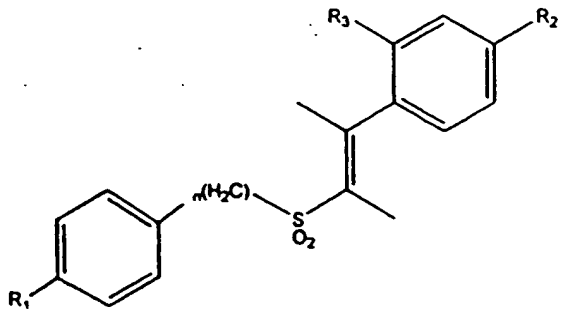
R_1 and R_2 are independently selected from the group consisting of chlorine, fluorine and bromine; and

R_3 is selected from the group consisting of hydrogen and fluorine;

R_1 and R_2 may not both be chlorine when R_3 is hydrogen; and

R_1 may not be chlorine when R_2 is fluorine and R_3 is hydrogen in the same compound.

According to another embodiment of the invention, a pharmaceutical composition is provided comprising a pharmaceutically acceptable carrier and a compound of formula II



II

wherein

n is zero or one;

R_1 is selected from the group consisting of hydrogen, chlorine, fluorine and bromine;

R_2 is selected from the group consisting of hydrogen, chlorine, fluorine, bromine, methyl and methoxy; and

R_3 is selected from the group consisting of hydrogen, chlorine and fluorine;

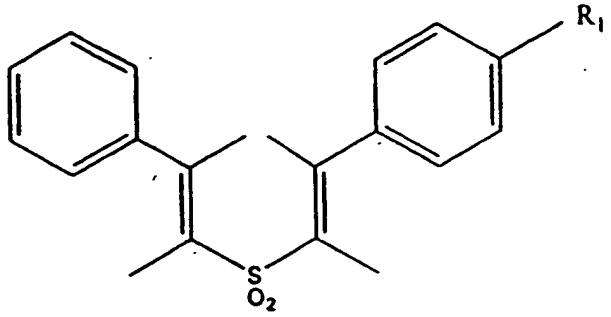
provided,

R_2 may not be methyl or methoxy when R_1 and R_3 are both hydrogen and n is zero or one; and

R_1, R_2 and R_3 may not all be hydrogen when n is one.

According to a preferred embodiment, the pharmaceutical composition comprises a pharmaceutically acceptable carrier and a compound of the formula II, wherein R_3 is hydrogen, and R_1 and R_2 are independently selected from the group consisting of chlorine, fluorine and bromine.

According to another embodiment of the invention, a pharmaceutical composition is provided comprising a pharmaceutically acceptable carrier and a compound of the formula III



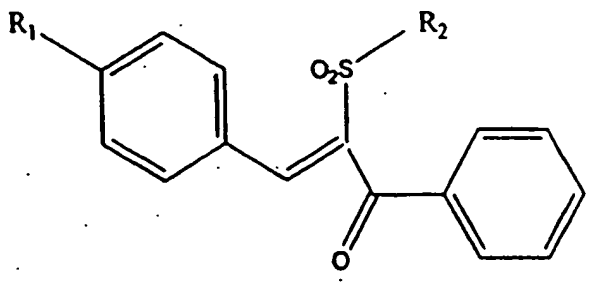
III

wherein

R_1 is selected from the group consisting of hydrogen, chlorine, fluorine and bromine.

According to another embodiment of the invention, a method of treating an individual for breast or prostate cancer is provided, comprising administering to said individual an effective amount of a compound according to formula II or formula III, alone or in combination with a pharmaceutically acceptable carrier. In another embodiment, a method of inhibiting growth of breast or prostate tumor cells in an individual afflicted with breast or prostate cancer is provided, comprising administering to said individual an effective amount of a compound according to formula III, alone or in combination with a pharmaceutically acceptable carrier. Furthermore, a method of inducing apoptosis of breast or prostate tumor cells in an individual afflicted with breast or prostate cancer is provided comprising administering to said individual an effective amount of a compound according to formula III, alone or in combination with a pharmaceutically acceptable carrier.

The invention also relates to a pharmaceutical composition and therapeutic methods as described above, wherein the compound is of the formula IV:



IV

wherein

R_1 is selected from the group consisting of fluorine and bromine, and R_2 is selected from the group consisting of 2-chlorophenyl, 4-chlorophenyl, 4-fluorophenyl and 4-nitro.

Description of the Figures

Figs. 1A and 1B are bar graphs of the effect of compounds E-2,4-difluorostyryl-4-fluorobenzyl sulfone (FRI-2), E-4-fluorostyryl 4-bromobenzyl sulfone (FRI-6), E-4-bromostyryl 4-fluorobenzyl sulfone (FRI-7), E-4-fluorostyryl 4-chlorobenzyl sulfone (FRI-20) and E-4-chlorostyryl 4-chlorobenzyl sulfone (FRI-22) on NIH3T3, MCF7, BT-20 and LnCaP cells. Cells were treated with the compounds at 2.5 μM (Fig. 1A) or 5.0 μM (Fig. 1B) concentration and cell viability was determined after 48 hours by Trypan blue exclusion method. Each data point represents the average of three independent experiments. The standard deviation did not exceed 10%.

Fig. 2A is a bar graph of the concentration dependent inhibition of MCF7, BT20, LnCaP and NIH3T3 cells by treatment with FRI-20. The cells were treated with 0, 250 nM, 500 nM, 1 μM, 2.5 μM and 5.0 μM FRI-20 for 48 hours. The percentage of living cells was determined by Trypan blue exclusion. The mean of three independent experiments is shown.

Fig. 2B is a bar graph of the viability of MCF7, BT20, LnCaP and NIH3T3 cells after treatment with FRI-20 at different time periods. All the cells were treated with FRI-20 at 2.5 μM, and the number of viable cells was determined at 12, 24, 48, and 72 hours by Trypan blue exclusion. The mean of three independent experiments is shown.

Fig. 3A is a plot of the activity of compound FRI-20 on the normal cell lines NIH3T3, HeLa and HFL; the estrogen receptor-positive breast tumor cell lines MCF-7 and 361; the estrogen receptor-negative breast tumor cell lines SKBR-3, 435 and BT-20). Fig. 3B is similar to Fig. 3A except the treated cells comprise the androgen-dependent prostate cell line LnCaP, and the androgen independent prostate cell lines DU-145 and PC-3). All cells were treated with 2.5 and 5.0 μM

concentration of FRI-20 and assayed for cell viability after 48 hours by Trypan blue exclusion. The mean of three experiments is shown. Variance did not exceed 10%.

Fig. 4 comprises a series of blots of the cell cycle analysis of FRI-20-treated or control-treated LnCaP cells. LnCaP cells were treated with 120 ml of DMSO (control cells) or 2.5 μM FRI-20 in 10 ml of DMSO. Cells were harvested 6. 12. 24 and 48 hours following treatment and stained with propidium iodide and subjected to flow cytometry.

Fig. 5 is an SDS-PAGE autoradiograph of the effect of FRI-20 on ERK/MAPK activity. FRI-20-treated LnCaP. MCF-7 and NIH3T3 cells. along with DMSO-treated cells (control). were processed for ERK/MAPK immune complex kinase essay using myelin basic protein (MBP) as a substrate. The activity of ERK-2 toward MBP was then assayed in the presence of [γ^{32}P]ATP. The phosphorylated MBP was separated on 12% SDS-PAGE and visualized by autoradiography.

Fig. 6 is blot of the distribution of ERK-2 and JNK/SAPK proteins in NIH3T3. LnCaP and MCF-7 cells. Lysates of cultured cells containing 100 mg of proteins were loaded per lane. Following electrophoresis and transfer to a polyvinylidene membrane. proteins were blotted against ERK-2 and JNK-2 polyclonal antibodies and visualized by chemiluminescence.

Fig. 7 is an SDS-PAGE autoradiograph of the effect of FRI-20 on JNK/SAPK activity. JNK was immunoprecipitated from 100 mg of cultured cell lysates with JNK polyclonal antibody, and an immune complex kinase assay was carried out using GST-c-Jun (1-79) as a substrate. The phosphorylated proteins were separated by SDS-PAGE and visualized by autoradiography. The experiment was repeated three times with similar results.

Detailed Description of the Invention

According to the present invention, certain styryl sulfone derivatives affect the MAPK signal transduction pathway, thereby affecting tumor cell growth and viability. The compounds inhibit the growth and proliferation of breast and prostate tumor cells in a dose-dependent manner, without affecting normal cell growth. This cell growth inhibition is associated with regulation of the ERK and JNK types of MAPK. The ability of the styryl sulfones to regulate these MAPKs and

induce cell growth arrest is dictated by the nature and position of the functional groups present in the compound.

Treatment of breast and prostate tumor cells with the styryl sulfone compounds of the invention leads to inhibition of cell proliferation and induction of apoptotic cell death. The effect is observed for estrogen receptor (ER) positive as well as estrogen receptor negative cells, although once breast cancer cell line tested, cell line 361, showed considerable resistance to styryl sulfones. Inhibition of cell proliferation and induction of apoptotic cell death is also observed for androgen-dependent as well as androgen-independent prostate tumor cells, although the former are considerably more sensitive to the styryl sulfones.

Tumor cells treated with the compounds of the invention accumulate in the G2/M phase of the cell cycle. As the cells exit the G2/M phase, they appear to undergo apoptosis. Treatment of normal cells with the styryl sulfones fails to produce a similar effect on cell cycle progression. Normal cells exhibit normal cell cycle progression in the presence and absence of styryl sulfone drug.

Both cells treated with the styryl sulfone compounds of the invention and untreated cells exhibit similar levels of intracellular ERK-2, but the biochemical activity of ERK-2, as judged by its ability to phosphorylate the substrate myelin basic protein (MBP), is considerably diminished in drug-treated cell compared to untreated cells. In prostate tumor cells, FR-20, a preferred compound of the invention, reduced the phosphorylation status of MBP by more than 80% compared to mock-treated cells. Western blot analysis of the drug and mock-treated cell lysates with ERK-2 antibody shows the same amount of protein in both lysates, indicating that higher levels of phosphorylated MBP in mock treated cells was not due to an unequal quantity of ERK-2 protein in the lysates. These results suggest that the styryl sulfones of the present invention block the phosphorylating capacity of ERK-2.

The styryl sulfones of the present invention enhance the ability of JNK to phosphorylate c-Jun protein compared to mock-treated cells. Without wishing to be bound by any theory, this result suggests that the styryl sulfones may be acting like pro-inflammatory cytokines or UV light, activating the JNK pathway, which in turn may switch on genes responsible for cell growth inhibition and apoptosis.

Synthesis f Styryl Sulfones

The compounds of the invention are characterized by cis-trans isomerism resulting from the presence of one or more double bonds. The compounds are named according to the Cahn-Ingold-Prelog system, the IUPAC 1974 Recommendations, Section E: Stereochemistry, in *Nomenclature of Organic Chemistry*, Pergamon, Elmsford, NY, 1979 (the "Blue Book"). See also, March, *Advanced Organic Chemistry*, John Wiley & Sons, Inc., New York, NY, 4th ed., 1992, p. 127-138. Stearic relations around a double bond are designated as "*Z*" or "*E*".

(*E*)-styryl and benzyl sulfones are prepared by Knoevenagel condensation of aromatic aldehydes with active methylene molecules such as aryl, benzyl, styryl sulfonyl acetic acids, phenacyl aryl sulfones and sulfonyl diacetic acid. The procedure is described by Reddy *et al.*, *Acta. Chim. Hung.* 115:269 (1984); Reddy *et al.*, *Sulfur Letters* 13:83 (1991); Reddy *et al.*, *Synthesis* 322 (1984); and Reddy *et al.*, *Sulfur Letters* 7:43 (1987), the entire disclosures of which are incorporated herein by reference. (*Z*)-benzyl and (*Z*)-styryl sulfones are synthesized by the nucleophilic addition of aromatic and aliphatic thiols to phenyl acetylene, and subsequent oxidation of the product with 30% hydrogen peroxide.

Preparation of Benzylsulfonyl and Arylsulfonyl Acetic Acids

Aryl and benzylsulfonyl acetic acids are the starting compounds for the synthesis of (*E*)-styryl aryl and (*E*)-styryl benzyl sulfones. Arylsulfonyl acetic acids may be prepared by the condensation of sodium aryl sulfinate with chloroacetic acid at alkaline pH. An alternate method for the synthesis of same compounds involves oxidizing the products obtained by the condensation of sodium arylthiolate with chloroacetic acid.

Benzylsulfonyl acetic acids may be synthesized by 30% hydrogen peroxide oxidation of the condensation products of the condensation of benzyl chlorides with sodium thioglycollate. Alternatively, benzylsulfonyl acetic acids may be synthesized by 30% hydrogen peroxide oxidation of the products of the condensation of sodium salts of benzyl thiols with chloroacetic acids.

Synthesis of (*E*)-Styryl Aryl and (*E*)-Benzyl Sulfones

To prepare the (*E*)-styryl benzyl and (*E*)-styryl benzyl sulfones, a mixture of the appropriate sulfonylacetic acid (*e.g.*, 10 mmol), an aromatic aldehyde (*e.g.*, 10 mmol) and a catalytic amount of benzylamine in acetic acid (*e.g.*, 15 ml) is refluxed for 2-3 hours. After cooling, dry ether is added and the reaction mixture is refrigerated overnight. The ethereal solution is washed successively with a saturated solution of sodium hydrogen carbonate, sodium bisulfite, dilute hydrochloric acid and finally with water. Evaporation of the sodium sulfate dried ethereal solution gives solid products of (*E*)-styryl aryl or benzyl sulfones which may be recrystallized with 2-propanol or 95% ethanol.

Synthesis of (*Z*)-Styryl Aryl and (*Z*)-Styryl Benzyl Sulfones

(*Z*)-Styryl aryl and (*Z*)-styryl benzyl sulfones may be prepared by the addition of sodium arylthiolate or benzylthiolate prepared from appropriate thiol (*e.g.*, 10 mmol) and sodium hydroxide (*e.g.*, 20 mmol) to freshly distilled phenylacetylene in methanol. The mixture is refluxed for 24 hours and poured onto crushed ice. The (*Z*)-styryl aryl and (*Z*)-styryl benzyl sulfides are oxidized with 30% hydrogen peroxide to provide (*Z*)-styryl aryl and (*Z*)-styryl benzyl sulfones, respectively.

Synthesis of (*E*),(*E*)- and (*E*),(*Z*)-bis(Styryl) Sulfones

(*E*),(*E*)-bis(styryl) sulfones may be prepared by the condensation of sulfonyl diacetic acid with aromatic aldehydes in the presence of benzylamine as catalyst. The reaction mixture is refluxed for 2 hours in glacial acetic acid. After cooling, absolute ether is added to the reaction mixture, which is washed successively with saturated solution of sodium bicarbonate, sodium bisulfite, dilute hydrochloric acid and water. Evaporation of the dried etherial layer yields (*E*),(*E*)-bis(styryl) sulfones.

(*Z*),(*E*)-bis(styryl) sulfones may be prepared by mixing a solution of (*Z*)-styrylsulfonyl acetic acid in glacial acetic acid with araldehyde and benzylamine. The solution is boiled for 3 hours. The reaction mixture is cooled and dry ether is added. Any product separated is filtered. The filtrate is diluted with more ether and

washed with saturated solution of sodium hydrogen carbonate, sodium bisulfite, dilute hydrochloric acid and water. The ether layer is separated, dried and evaporated to give (*Z*),(*E*)-bis(styryl) sulfones.

Therapeutic Administration

The styryl sulfones of the invention may be administered in the form of a pharmaceutical composition, in combination with a pharmaceutically acceptable carrier. The active ingredient in such formulations may comprise from 0.1 to 99.99 weight percent. By "pharmaceutically acceptable carrier" is meant any carrier, diluent or excipient which is compatible with the other ingredients of the formulation and to deleterious to the recipient.

The compounds of the invention may be administered to individuals (mammals, including animals and humans) afflicted with breast or prostate cancer. The compounds may be administered by any route, including oral and parenteral administration. Parenteral administration includes, for example, intravenous, intramuscular, intraarterial, intraperitoneal, intranasal, rectal, or subcutaneous administration. The active agent is preferably administered with a pharmaceutically acceptable carrier selected on the basis of the selected route of administration and standard pharmaceutical practice.

The active agent may be formulated into dosage forms according to standard practices in the field of pharmaceutical preparations. See Gennaro Alphonso, ed., *Remington's Pharmaceutical Sciences*, 18th Ed., (1990) Mack Publishing Co., Easton, PA. Suitable dosage forms may comprise, for example, tablets, capsules, solutions, parenteral solutions, troches, suppositories, or suspensions.

For parenteral administration, the active agent may be mixed with a suitable carrier or diluent such as water, an oil, saline solution, aqueous dextrose (glucose) and related sugar solutions, or a glycol such as propylene glycol or polyethylene glycol. Solutions for parenteral administration preferably contain a water soluble salt of the active agent. Stabilizing agents, antioxidizing agents and preservatives may also be added. Suitable antioxidizing agents include sulfite, ascorbic acid, citric acid and its salts, and sodium EDTA. Suitable preservatives include benzalkonium chloride, methyl- or propyl-paraben, and chlorbutanol.

For oral administration, the active agent may be combined with one or more solid inactive ingredients for the preparation of tablets, capsules, or other suitable oral dosage forms. For example, the active agent may be combined with carboxymethylcellulose calcium, magnesium stearate, mannitol and starch, and then formed into tablets by conventional tableting methods.

The specific dose of compound according to the invention to obtain therapeutic benefit will, of course, be determined by the particular circumstances of the individual patient including, the size, weight, age and sex of the patient, the nature and stage of the disease, the aggressiveness of the disease, and the route of administration. For example, a daily dosage of from about 0.05 to about 50 mg/kg day may be utilized. Higher or lower doses are also contemplated.

The practice of the invention is illustrated by the following non-limiting examples.

Procedure 1

General procedure for Synthesis of Styryl and Benzyl Arylsulfones

To a solution of (8 g, 0.2 mol) sodium hydroxide in methanol (200 ml), appropriate thiophenol or benzyl mercaptan (0.1 mol) is added slowly. Then chloroacetic acid (0.1 mol) is added in portions and the reaction mixture is refluxed for 2-3 hours. The cooled contents are poured onto crushed ice and neutralized with dilute hydrochloric acid (200 ml). The resulting aryl and benzylthioacetic acids (0.1 mol) are subjected to oxidation with 30% hydrogen peroxide (0.12 mol) in glacial acetic acid (25 ml) by refluxing for 1-2 hours. The contents are cooled and poured onto crushed ice. The separated solid is recrystallized from hot water to give pure aryl and benzylsulfonyl acetic acids.

A mixture of the appropriate aryl or benzylsulfonyl acetic acid (0.001 mol), an aromatic aldehyde (0.001 mol) and benzylamine (1 ml) in glacial acetic acid (15 ml) is reflexed for 2-3 hours. The contents are cooled and treated with dry ether (50 ml). Any product separated is collected by filtration. The filtrate is diluted with more ether and washed successively with a saturated solution of sodium bicarbonate (20 ml), sodium bisulfite (20 ml), dilute hydrochloric acid

(20 ml) and finally with water (35 ml). Evaporation of the dried ethereal layer yields a solid in many cases. However, in some cases a syrupy material separates and is solidified on treatment with 2-propanol. The purity of the compounds is checked by TLC (silica gel BDH, hexane/ethyl acetate 3:1).

Procedure 2

General procedure for Synthesis of (E)(E)- and (E)(Z)-bis(Styryl) Sulfones

To freshly distilled phenyl acetylene (51.07 g, 0.5 mol) is added sodium thioglycollate prepared from thioglycolic acid (46 g, 0.5 mol) and sodium hydroxide (40 g, 1 mol) in methanol (250 ml). The mixture is refluxed for 24 hours and poured onto crushed ice (500 ml) after cooling. The styrylthioacetic acid, formed after neutralization with dilute hydrochloric acid (250 ml), is filtered and dried; yield 88 g (90%); m.p. 84-86°C.

The styrylthioacetic acid is then oxidized to styrylsulfonylacetic acid as follows. A mixture of styrylthioacetic acid (5 g, 25 mmol) in glacial acetic acid (35 ml) and 30% hydrogen peroxide (15 ml) is heated under reflux for 60 minutes and the mixture is poured onto crushed ice (200 ml) after cooling. The compound separated is filtered and recrystallized from hot water to give white crystalline flakes of (Z)-styrylsulfonylacetic acid; yield 2.4 g (41%); m.p. 150-51°C.

A solution of (Z)-styrylsulfonylacetic acid (2.263 g, 10 m mol) in glacial acetic acid (6 ml) is mixed with an aromatic aldehyde (10 mmol) and benzylamine (0.2 ml) and refluxed for 3 hours. The reaction mixture is cooled, treated with dry ether (50 ml), and any product separated is collected by filtration. The filtrate is diluted with more ether and washed successively with a saturated solution of sodium hydrogen carbonate (15 ml), sodium bisulfite (15 ml), dilute hydrochloric acid (20 ml) and finally with water (30 ml). Evaporation of the dried ethereal layer yields (E)(Z)-bis(styryl)sulfones.

(E),(E)-bis(styryl)sulfones are prepared following the same procedure as described above with exception that sulfonyldiacetic acid is used in place of (Z)-styrylsulfonylacetic acid, and twice the amount of aromatic aldehyde (20 mmol) is used.

Procedure 3

General Procedure for the Synthesis of 2-(Arylsulfonyl)-1-phenyl-3-aryl-2-propen-1-ones

These compounds are synthesized by two methods which employ different reaction conditions, solvents and catalysts.

Method 1: Phenacyl aryl sulfones are made by refluxing α-bromoacetophenones (0.05 mol) and sodium arylsulfinates (0.05 mol) in absolute ethanol (200 ml) for 6-8 hours. The product which separates on cooling is filtered and washed several times with water to remove sodium bromide. The product is then recrystallized from ethanol: phenacyl-phenyl sulfone, m.p. 90-91°C; phenacyl-p-fluorophenyl sulfone, m.p. 148-149°C; phenacyl-p-bromophenyl sulfone, m.p. 121-122°C; phenacyl-p-methoxyphenyl sulfone, m.p. 104-105°C; p-nitrophenacyl-phenyl sulfone, m.p. 136-137°C.

A solution of phenacyl aryl sulfone (0.01 mol) in acetic acid (10 ml) is mixed with an araldehyde (0.01 mol) and benzylamine (0.02 ml) and refluxed for 3 hours. The solution is cooled and dry ether (50 ml) is added. The ethereal solution is washed successively with dilute hydrochloric acid, aqueous 10% NaOH, saturated $NaHSO_3$ solution and water. Evaporation of the dried ethereal layer gives a solid product which is purified by recrystallization.

Method 2: Dry tetrahydrofuran (200 ml) is taken in a 500 ml conical flask flushed with nitrogen. To this, a solution of titanium (IV) chloride (11 ml, 0.01 mol) in absolute carbon tetrachloride is added dropwise with continuous stirring. The contents of the flask are maintained at -20°C throughout the course of the addition. A mixture of phenacyl aryl sulfone (0.01 mol) and aromatic aldehyde (0.01 mol) is added to the reaction mixture and pyridine (4 ml, 0.04 mol) in tetrahydrofuran (8 ml) is added slowly over a period of 1 hour. The contents are stirred for 10-12 hours, treated with water (50 ml) and then ether (50 ml) is added. The ethereal layer is separated and washed with 15 ml of saturated solutions of 10%

sodium hydroxide, sodium bisulfite and brine. The evaporation of the dried ethereal layer yields 2-(arylsulfonyl)-1-phenyl-3-aryl-2 propen-1-ones.

Example 1

E-styryl phenyl sulfone

A solution of phenyl sulfonylacetic acid (0.01 mol) and benzaldehyde (0.01 mol) was subjected to the Procedure 1. The title compound was obtained in 68-72% yield.

Example 2

E-4-chlorostyryl phenyl sulfone

A solution of phenyl sulfonylacetic acid (0.01 mol) and 4-chlorobenzaldehyde (0.01 mol) was subjected to Procedure 1. The title compound was obtained in 78-80% yield.

Example 3

E-2,4-dichlorostyryl phenyl sulfone

A solution of phenyl sulfonylacetic acid (0.01 mol) and 2,4-dichlorobenzaldehyde (0.01 mol) was subjected to Procedure 1. The title compound was obtained in 60-65% yield.

Example 4

E-4-bromostyryl phenyl sulfone

A solution of phenyl sulfonylacetic acid (0.01 mol) and 4-bromobenzaldehyde (0.01 mol) was subjected to Procedure 1. The title compound was obtained in 78-80% yield.

Example 5

E-4-chlorostyryl 4-chlorophenyl sulfone

A solution of 4-chlorophenyl sulfonylacetic acid (0.01 mol) and 4-chlorobenzaldehyde (0.01 mol) was subjected to Procedure 1. The title compound was obtained in 70-72% yield.

Example 6

E-4-methylstyryl 4-chlorophenyl sulfone

A solution of 4-chlorophenyl sulfonylacetic acid (0.01 mol) and 4-methylbenzaldehyde (0.01 mol) was subjected to Procedure 1. The title compound was obtained in 60-64% yield.

Example 7

E-4-methoxystyryl 4-chlorophenyl sulfone

A solution of 4-chlorophenyl sulfonylacetic acid (0.01 mol) and 4-methoxybenzaldehyde (0.01 mol) was subjected to Procedure 1. The title compound was obtained in 68-70% yield.

Example 8

E-4-bromostyryl 4-chlorophenyl sulfone

A solution of 4-chlorophenyl sulfonylacetic acid (0.01 mol) and 4-bromobenzaldehyde (0.01 mol) was subjected to Procedure 1. The title compound was obtained in 80% yield.

Example 9

E-2-chlorostyryl benzyl sulfone

A solution of benzyl sulfonylacetic acid (0.01 mol) and 2-chlorobenzaldehyde (0.01 mol) was subjected to Procedure 1. The title compound was obtained in 72% yield.

Example 10

E-4-chlorostyryl benzyl sulfone

A solution of benzyl sulfonylacetic acid (0.01 mol) and 4-chlorobenzaldehyde (0.01 mol) was subjected to Procedure 1. The title compound was obtained in 78% yield.

Example 11

E-4-fluorostyryl 4-chlorobenzyl sulfone

A solution of 4-chlorobenzyl sulfonylacetic acid (0.01 mol) and 4-fluorobenzaldehyde (0.01 mol) was subjected to Procedure 1. The title compound was obtained in 72% yield.

Example 12

E-4-chlorostyryl 4-chlorobenzyl sulfone

A solution of 4-chlorobenzyl sulfonylacetic acid (0.01 mol) and 4-chlorobenzaldehyde (0.01 mol) was subjected to Procedure 1. The title compound was obtained in 80% yield.

Example 13

E-4-fluorostyryl 4-fluorobenzyl sulfone

A solution of 4-fluorobenzyl sulfonylacetic acid (0.01 mol) and 4-fluorobenzaldehyde (0.01 mol) was subjected to Procedure 1. The title compound was obtained in 73% yield.

Example 14

E-2,4-difluorostyryl 4-fluorobenzyl sulfone

A solution of 4-fluorobenzyl sulfonylacetic acid (0.01 mol) and 2,4-difluorobenzaldehyde (0.01 mol) was subjected to Procedure 1. The title compound was obtained in 68% yield.

Example 25

2-[(2-chlorophenyl)sulfonyl]-1-phenyl-3-(4-bromophenyl)-2-propen-1-one

A solution of phenacyl-2-chlorophenyl sulfone (0.01 mol) and 4-bromo benzaldehyde (0.01 mol) was subjected to Method 1 of Procedure 3. The title compound was obtained in 66% yield.

Example 26

2-[(4-chlorophenyl)sulfonyl]-1-phenyl-3-(4-bromophenyl)-2-propen-1-one

A solution of phenacyl-4-chlorophenyl sulfone (0.01 mol) and 4-bromo benzaldehyde (0.01 mol) was subjected to Method 1 of Procedure 3. The title compound was obtained in 60% yield.

Example 27

2-[(2-nitrophenyl)sulfonyl]-1-phenyl-3-(4-bromophenyl)-2-propen-1-one

A solution of phenacyl-2-nitrophenyl sulfone (0.01 mol) and 4-bromo benzaldehyde (0.01 mol) was subjected to Method 1 of Procedure 3. The title compound was obtained in 56% yield.

Example 28

Tumor Cell Growth Inhibition by Styryl Sulfones

A. Cells.

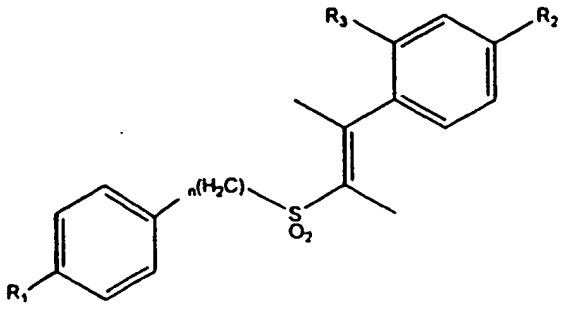
The effect of the styryl sulfones on the growth of normal and tumor cells of breast and prostate was examine utilizing four cell lines, NIH3T3, MCF-7, BT-20 and LnCap. NIH/3T3 cells represent normal fibroblasts while LnCap is an androgen-dependent prostate tumor cell line. MCF-7 is an estrogen-responsive breast tumor cell line, while BT-20 is an estrogen-unresponsive breast tumor cell line. MCF-7 and BT-20 were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum supplemented with penicillin and streptomycin. LnCaP were cultured in RPMI with 10% fetal bovine serum containing penicillin and streptomycin. NIH3T3 cells were grown in DMEM containing 10% calf serum

supplemented with penicillin and streptomycin. All cell cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2.

B. Treatment with Styryl Sulfones and Viability Assay

Cells were treated with test compound at 2.5 μM or 5.0 μM concentration and cell viability was determined after 48 hours by the Trypan blue exclusion method. The compounds identified in Tables 1, 2 and 3 inhibited cell growth and induced cell death, to varying degrees. The tables list the percent viable LnCap and MCF-7 cells treated with 5.0 μM compound.

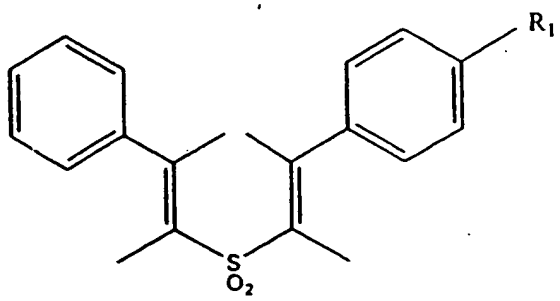
Table 1



II

Example	n	R_1	R_2	R_3	% viable LnCaP and MCF-7 cells
1	0	H	H	H	89
2	0	H	Cl	H	90
3	0	H	Cl	Cl	88
4	0	H	Br	H	68
5	0	Cl	Cl	H	64
6	0	Cl	CH_3	H	92
7	0	Cl	OCH_3	H	90
8	0	Cl	Br	H	69
9	1	H	H	Cl	94
10	1	H	Cl	H	87
11	1	Cl	F	H	6
12	1	Cl	Cl	H	49
13	1	F	F	H	43
14	1	F	F	F	56
15	1	Br	F	H	7
16	1	Br	Br	H	51
17	1	F	Br	H	42
18	1	Br	Cl	H	7
19	1	Cl	Br	H	20

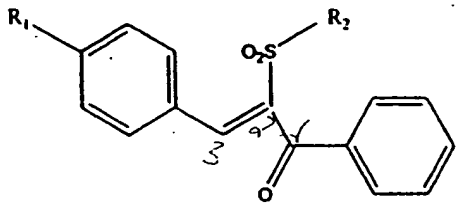
Table 2



III

Example	R_1	% viable LnCaP and MCF-7 cells
20	F	76
21	Br	68
22	Cl	72

Table 3



IV

Example	R_1	R_2	% viable LnCaP and MCF-7 cells
23	F	4-fluorophenyl	76
24	F	2-chlorophenyl	64
25	Br	2-chlorophenyl	72
26	Br	4-chlorophenyl	58
27	Br	2-nitro	74

Five of the more active compounds which exhibited the highest activity were designated as FRI-2 (E-2,4-difluorostyrl-4-fluorobenzyl sulfone), FRI-6 (E-4-fluorostyryl 4-bromobenzyl sulfone), FRI-7 (E-4-bromostyryl 4-fluorobenzyl sulfone), FRI-20 (E-4-fluorostyryl 4-chlorobenzyl sulfone) and FRI-22 (E-4-chlorostyryl 4-chlorobenzyl sulfone). These compounds were found to substantially inhibit the growth and induce the death of LnCaP, BT-20 and MCF-7 cells at 2.5 mM (Figure 1A) and 5.0, mM (Figure 1B), after 48 hours of treatment with the compounds. Under identical conditions, more than 80% of NIH3T3 cells were viable after 48 hours incubation (Figure 1A and 1B). E-4-chlorostyryl 4-bromobenzyl sulfone and E-4-bromostyryl 4-chlorobenzyl sulfone were also highly active.

C. Dose-Dependency Assay

The dose dependency of the styryl sulfone was established by treating the cells with FRI-20, one of the five most active compounds. NIH3T3, MCF-7, BT-20 and

cell lines); MCF-7 and 361 (estrogen-receptor negative breast tumor cell lines); BT-20, 435 and SKBR-3 (estrogen-receptor positive breast tumor cell lines); LnCaP (androgen sensitive prostate tumor cell line); PC-3 and DU-145 (androgen insensitive prostate tumor cell line).

B. Treatment with FRI-20 and Viability Assay.

The cells were grown as in Example 22, A. FR-20 was dissolved in DMSO and added to the cells at 2.5 μM and 5.0 μM concentration. To control cells, DMSO was added equivalent to the volume of solvent (DMSO) present at the highest concentration of the compound. The activity of the compound was evaluated after 48 hours by Trypan blue exclusion. NIH3T3 and HFL cells were found to maintain a percent viability of 85-90% at 2.5 and 5.0 μM concentration. Of the seven breast tumor cell lines treated with FRI-20 compound, MCF-7, HTB126, T470 and 435 cells showed very high mortality with less than 25% and 10% viability at 2.5 and 5.0 μM concentrations of the drug (Figure 3A). Nearly 50% of SKBR-3 and BT-20 cells were dead at 2.5 μM and 75% at 5.0 μM concentration of the compound. The 361 breast tumor cell line, on the other hand showed considerable resistance to FRI-20 with 50-75% of cells being viable at 2.5 and 5.0 μM concentration. FRI-20 had profound effect on the viability of androgen-dependent LnCaP prostate tumor cell line when compared to androgen-independent DU-145 and PC-3 prostate cell lines. At 2.5 mM FRI-20, 80% of LnCaP, 40% of PC-3 and 20% of DU-145 cells were killed. At 5.0 mM FRI-20, 72% of LnCaP, 47% of PC-3 and 40% of DU-145 were killed (Figure 3B).

Example 30

Effect of FRI-20 on Cell Cycle Regulation

The androgen-dependent prostate tumor cell line LnCaP was grown as in Ex. 22, A. and treated with 2.0 μM FRI-20 dissolved in DMSO or with equivalent amounts (10 ml) of DMSO alone. Cells were harvested 6, 12, 24, and 48 hours following treatment and stained with propidium iodide and subjected to flow cytometry (FACS) for analysis of DNA content. As shown in Fig. 4, the addition of FR-20 to the culture medium results in the accumulation of cells in the G2/M phase

of the cell cycle and as the cells exit this phase of the cell cycle, they appeared to undergo apoptosis. Cells treated with DMSO alone failed to exhibit such an arrest in the G2/M phase of the cell cycle, suggesting that the effects seen are associated with FRI-20 addition. Treatment of the normal cell lines NIH3T3 or HFL with FRI-20 failed to produce a similar effect on cell cycle progression. NIH3T3 and HFL exhibited normal cell cycle progression in the presence and absence of drug.

Example 31

Effect of FRI-20 on MPK Pathway

A. Immune Complex ERK-2 Assay.

To examine the effects of FRI-20 on the MAPK pathway, NIH3T3, LnCaP and MCF-7 cells were incubated with FRI-20 at a concentration 2.5 mM for 48 hours. Following incubation of cells in the presence and absence of FRI-20, the cells were lysed using ERK lysis buffer containing 20 mM HEPES (pH 7.4), 50 mM β-glycerophosphate, 0.5% Triton X-100, 2 mM $MgCl_2$, 1 mM EGTA, 1 mM dithiothreitol, 2 μg/ml leupeptin, 2 μg/ml aprotinin, 100 μM phenylmethylsulfonyl fluoride, and 1 mM benzamidine. ERK-2 in 100 mg of cell lysate was immunoprecipitated by incubating lysate protein with 1 mg of ERK-2 polyclonal antibody (antibody sc-154 to ERK2 is from Santa Cruz Biotechnology, Inc.) for one hour followed by an additional incubation of 20 μl of protein A-Sepharose (Pharmacia) for one hour. The immune complex-bound protein A-Sepharose beads were washed twice with lysis buffer and twice with ERK/MAPK buffer containing 20 mM HEPES (pH 7.4), 50 mM β-glycerophosphate, 10 mM $MgCl_2$, 1 mM EGTA, 1 mM dithiothreitol, and 100 MM Na_3VO_4.

The immunoprecipitates were then tested for MAP kinase activity by an *in vitro* assay which utilizes myelin basic proteins (MBP) as a substrate for ERK-2 in the presence of [γ-^{32}P] ATP. Accordingly, the beads were resuspended in 40 μl of MAPK buffer containing 100 μM [γ-^{32}P] ATP (5000 cpm/pmol), and the kinase assay was carried out for 20 minutes at 30°C using 5 μg of MBP as substrate. The reaction was stopped by the addition of Laemmli's buffer followed by the boiling of the samples for 3 minutes. The proteins were resolved on 12% SDS-PAGE; the gel

was dried, and an autoradiogram was developed. The results show that both drug-treated and untreated cells exhibit similar levels of intracellular ERK-2, but the biochemical activity of ERK-2, as judged by its ability to phosphorylate MBP, was considerably diminished in drug-treated cells compared to cells treated with DMSO alone. In prostate tumor cells, FRI-20 reduced the phosphorylation status of MBP by more than 80% compared to mock-treated cells (Figure 5).

B. Western Blot Analysis.

Cell lysates of FRI-20-treated cells were prepared for Western Blot analysis as follows. NIH3T3, LnCaP or MCF-7 cells were seeded at a density of $2X10^5$ cells/per well in a six-well plate and allowed to grow for 24 hours. Fresh medium was added to each well 2 hours before treatment with FRI-20. The compound was dissolved in DMSO to make a 2 mM stock solution and added to the medium (2 ml) to obtain a final concentration of 2.5 and 5.0 μM. After 48 hours at 37°C, the cells were washed twice with 10 ml of ice cold phosphate-buffered saline and harvested in 400 μl of lysis buffer containing 25 mM HEPES (pH 7.6), 0.1% Triton X-100, 300 mM NaCl, 1.5 mM $MgCl_2$, 20 mM β-glycerophosphate, 100 μM Na_3VO_4, 0.2 mM EDTA, 0.5 mM dithiothreitol, 2 μg/ml aprotinin, 2 μg/ml leupeptin, 100 μM phenylmethylsulfonyl chloride and 1 mM benzamidine. The cell lysates were kept on ice for 30 minutes and centrifuged for 10 minutes in a microcentrifuge (16000 X g). The cell lysates were separated from the debris and normalized for protein content.

Western Blot analysis was carried out on the drug- and mock-treated cell lysates with ERK-2 antibody. Equal amounts of total protein (100 µg) were loaded in each lane of a SDS-PAGE gel (10-12%) and transferred to Immobilon-P (Millipore, USA). Following transfer, membranes were blocked in 3% milk, then probed with ERK-2 and JNK-1 rabbit polyclonal antibodies (Santa Cruz Biotechnology Inc., Santa Cruz, CA) and then probed with horseradish peroxidase linked donkey anti-rabbit Ig secondary antibody (Amersham) (1:10000 dilution). The antibody was detected using the ECL Western blotting analysis kit (Amersham) following the manufacturer's instructions. The western blot analysis of the drug- and

mock-treated cell lysates with ERK-2 antibody showed the same amount of protein in both lysates (Figure 6), indicating that higher levels of MBP phosphorylation in mock-treated cells was not due to an unequal quantity of ERK-2 protein in lysates. These results suggest that FRI-20 blocks the phosphorylating capability of ERK-2.

Example 32

Effect of FRI-20 on Stress-Activated Protein Activity

To further establish if the activity of stress activated protein kinases (SAPKs), of which JNK is a member, is compromised in the presence of FRI-20, cells (NIH3T3, MCF-7 or LnCaP) were treated with FRI-20 dissolved in DMSO or with DMSO alone. Forty-eight hours later, the cells were lysed with kinase buffer and the lysates used for estimation of the amount of JNK present in each lysate by western blot analysis using JNK polyclonal antibody. The biochemical activity of the JNK present in the FRI-20-treated and mock-treated cell lysates was also determined by immunoprecipitation of JNK followed by incubation with GST-c-Jun protein as a substrate for JNK in the presence of [γ-^{32}P]ATP.

Accordingly, JNK-1 in 100 mg of cell extracts was immunoprecipitated by incubating the lysate with 1 mg of JNK-1 polyclonal antibody (sc from Santa Cruz Biotechnology) for one hour followed by an additional incubation with 20 μl of protein A-Sepharose (Pharmacia) for one hour. The beads were washed twice with JNK lysis buffer (as described above) followed by two washes with JNK reaction buffer. The beads were resuspended in 40 μl of JNK buffer containing 20 mM [γ-^{32}P] ATP (5000 cpm/pmol), and the kinase reaction was carried out for 20 minutes at 30°C using 3 μg of purified GST-c-Jun(1-79) as substrate. The reaction was stopped, and the radioactivity in the phosphorylated GST-c-Jun protein was quantitated. The results show that the FRI-20 treatment enhanced the ability of JNK to phosphorylate recombinant GST-c-Jun protein by 60-80% compared to mock-treated cells (Figure 7).

JNK has been shown to be activated by treatment of cells with UV radiation, pro-inflammatory cytokines and environmental stress (Derijard *et al.*, *Cell* 1025 (1994)). The activated JNK binds to the amino terminus of c-jun and increases its transcriptional activity by phosphorylating at ser63 and ser73 (Adler *et al.*, *Proc.*

Natl. Acad. Sci. USA 89:5341 (1992); Kwok *et al.*, *Nature* 370:223 (1994)). Without wishing to be bound by any theory, the results demonstrated herein suggest that FRI-20 may act like a pro-inflammatory cytokine or UV light in activating the JNK pathway, which in turn may switch on genes responsible for cell growth inhibition and apoptosis.

Example 33

Comparison of FRI-20 and Cisplatin Anti-Tumor Activities

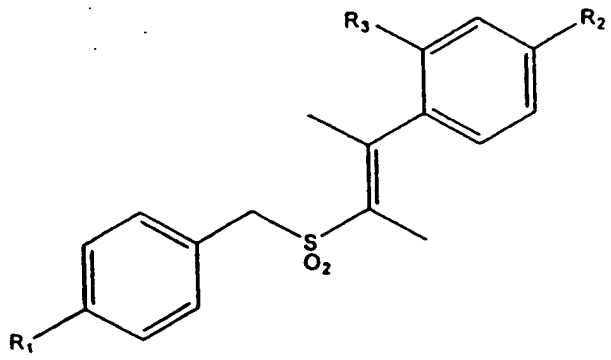
The killing effect of FR-20 on androgen-sensitive (LnCaP) and androgen insensitive (DU145) prostate tumor cells was compared to the effect of cisplatin (cis-diamminedichloroplatinum II), a widely used anti-prostate cancer agent. The cells were grown as in Example 26. FRI-20 or cisplatin was dissolved in DMSO and added to the cells at various concentrations. Viability was determined after 72 hours by the Trypan blue exclusion method. The concentration of FRI-20 required to completely kill LnCaP and DU145 cells was 2.5 μM and 5.0 μM, respectively. Under identical conditions, complete killing of LnCaP and DU145 cells by cisplatin required 25 μM and 15 μM concentrations, respectively. Thus, FRI-20 is at least tenfold more active than cisplatin in killing both hormone-dependent and hormone-independent prostate tumor cells.

All references cited with respect to synthetic, preparative and analytical procedures are incorporated herein by reference.

The present invention may be embodied in other specific forms without departing from the spirit or essential attributes thereof and, accordingly, reference should be made to the appended claims, rather than to the foregoing specification, as indication the scope of the invention.

CLAIMS

1. A compound of the formula I:



I

wherein

R_1 and R_2 are independently selected from the group consisting of chlorine, fluorine and bromine; and

R_3 is selected from the group consisting of hydrogen and fluorine;

R_1 and R_2 may not both be chlorine when R_3 is hydrogen; and

R_1 may not be chlorine when R_2 is fluorine and R_3 is hydrogen in the same compound.

2. A compound according to claim 1 wherein the compound is E-4-fluorostyryl 4-fluorobenzyl sulfone.

3. A compound according to claim 1 wherein the compound is E-2,4-difluorostyryl 4-fluorobenzyl sulfone.

4. A compound according to claim 1 wherein the compound is E-4-fluorostyryl 4-bromobenzyl sulfone.

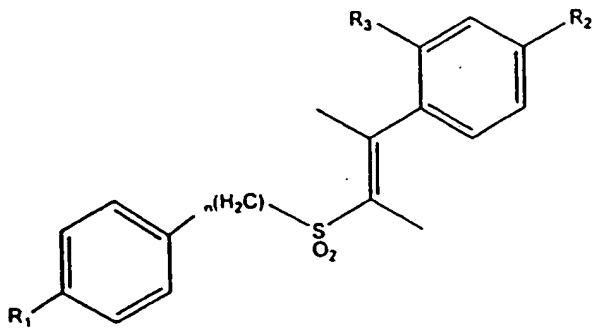
5. A compound according to claim 1 wherein the compound is E-4-bromostyryl 4-bromobenzyl sulfone.

6. A compound according to claim 1 wherein the compound is E-4-chlorostyryl 4-bromobenzyl sulfone.

7. A compound according to claim 1 wherein the compound is E-4-bromostyryl 4-chlorobenzyl sulfone.

8. A compound according to claim 1 wherein the compound is E-4-bromostyryl 4-fluorobenzyl sulfone.

9. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound of the formula II



II

wherein

n is zero or one;

R_1 is selected from the group consisting of hydrogen, chlorine, fluorine and bromine;

R_2 is selected from the group consisting of hydrogen, chlorine, fluorine, bromine, methyl and methoxy; and

R_3 is selected from the group consisting of hydrogen, chlorine and fluorine;

provided,

R_2 may not be methyl or methoxy when R_1 and R_3 are both hydrogen and n is zero or one; and

R_1, R_2 and R_3 may not all be hydrogen when n is one.

10. A composition according to claim 9 wherein the compound is E-4-bromostyryl phenyl sulfone.

11. A composition according to claim 9 wherein the compound is E-2,4-difluorostyryl 4-fluorobenzyl sulfone.

12. A composition according to claim 9, wherein R_3 is hydrogen, and R_1 and R_2 are independently selected from the group consisting of chlorine, fluorine and bromine.

13. A composition according to claim 12 wherein the compound is E-4-chlorostyryl 4-chlorophenyl sulfone.

14. A composition according to claim 12 wherein the compound is E-4-bromostyryl 4-chlorophenyl sulfone.

15. A composition according to claim 12 wherein the compound is E-4-fluorostyryl 4-chlorobenzyl sulfone.

16. A composition according to claim 12 wherein the compound is E-4-chlorostyryl 4-chlorobenzyl sulfone.

17. A composition according to claim 12 wherein the compound is E-4-fluorostyryl 4-fluorobenzyl sulfone.

18. A composition according to claim 12 wherein the compound is E-4-fluorostyryl 4-bromobenzyl sulfone.

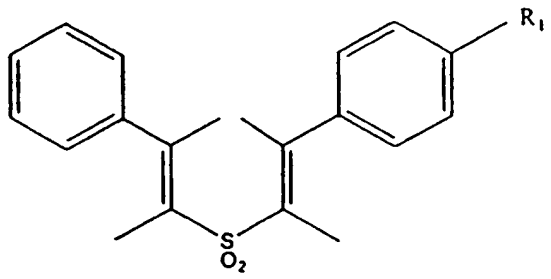
19. A composition according to claim 12 wherein the compound is E-4-bromostyryl 4-bromobenzyl sulfone.

20. A composition according to claim 12 wherein the compound is E-4-bromostyryl 4-fluorobenzyl sulfone.

21. A composition according to claim 12 wherein the compound is E-4-chlorostyryl 4-bromobenzyl sulfone.

22. A composition according to claim 12 wherein the compound is E-4-bromostyryl 4-chlorobenzyl sulfone.

23. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound of the formula III

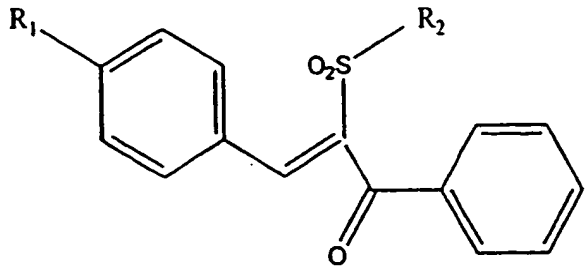


III

wherein

R_1 is selected from the group consisting of hydrogen, chlorine, fluorine and bromine.

24. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the compound of formula IV



IV

wherein

R_1 is selected from the group consisting of fluorine and bromine, and R_2 is selected from the group consisting of 2-chlorophenyl, 4-chlorophenyl, 4-fluorophenyl and 4-nitro.

25. A method of treating an individual for breast or prostate cancer comprising administering to said individual an effective amount of a pharmaceutical composition according to claim 9.

26. A method of inhibiting growth of breast or prostate tumor cells in an individual afflicted with breast or prostate cancer comprising administering to said individual a pharmaceutical composition according to claim 9.

27. A method of inducing apoptosis of breast or prostate tumor cells in an individual afflicted with breast or prostate cancer comprising administering to said individual a pharmaceutical composition according to claim 9.

28. A method of treating an individual for breast or prostate cancer comprising administering to said individual an effective amount of a pharmaceutical composition according to claim 23 or 24.

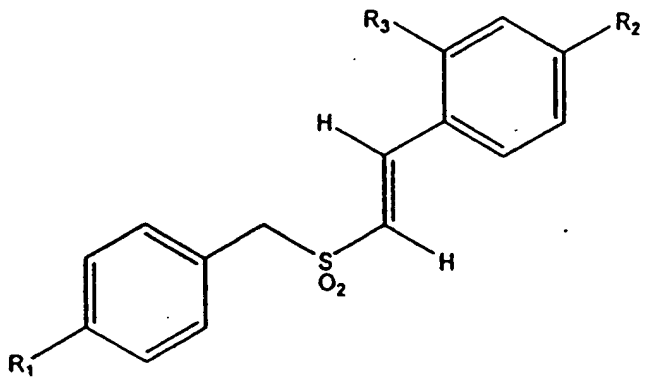
29. A method of inhibiting growth of breast or prostate tumor cells in an individual afflicted with breast or prostate cancer comprising administering to said individual a pharmaceutical composition according to claim 23 or 24.

30. A method of inducing apoptosis of breast or prostate tumor cells in an individual afflicted with breast or prostate cancer comprising administering to said individual a pharmaceutical composition according to claim 23 or 24.

AMENDED CLAIMS

[received by the International Bureau on 23 February1999 (23.02.99); original claims 1, 9, 23-30 amended; new claims 31-36 added; remaining claims unchanged (5 pages)]

1. A compound of the formula I:



I

wherein

R_1 and R_2 are independently selected from the group consisting of chlorine, fluorine and bromine; and

R_3 is selected from the group consisting of hydrogen and fluorine;

R_1 and R_2 may not both be chlorine when R_3 is hydrogen; and

R_1 may not be chlorine when R_2 is fluorine and R_3 is hydrogen in the same compound.

2. A compound according to claim 1 wherein the compound is E-4-fluorostyryl 4-fluorobenzyl sulfone.

3. A compound according to claim 1 wherein the compound is E-2,4-difluorostyryl 4-fluorobenzyl sulfone.

4. A compound according to claim 1 wherein the compound is E-4-fluorostyryl 4-bromobenzyl sulfone.

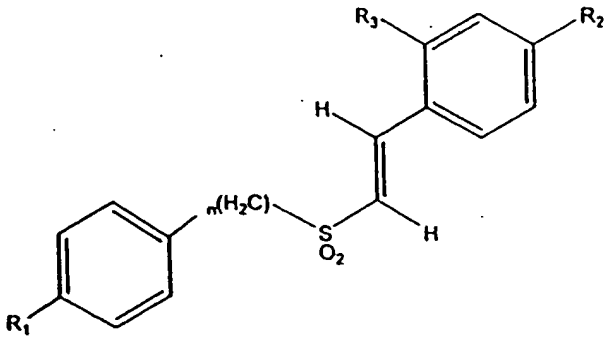
5. A compound according to claim 1 wherein the compound is E-4-bromostyryl 4-bromobenzyl sulfone.

6. A compound according to claim 1 wherein the compound is E-4-chlorostyryl 4-bromobenzyl sulfone.

7. A compound according to claim 1 wherein the compound is E-4-bromostyryl 4-chlorobenzyl sulfone.

8. A compound according to claim 1 wherein the compound is E-4-bromostyryl 4-fluorobenzyl sulfone.

9. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound of the formula II



II

wherein

n is zero or one;

R_1 is selected from the group consisting of hydrogen, chlorine, fluorine and bromine;

R_2 is selected from the group consisting of hydrogen, chlorine, fluorine, bromine, methyl and methoxy; and

R_3 is selected from the group consisting of hydrogen, chlorine and fluorine;

provided,

R_2 may not be methyl or methoxy when R_1 and R_3 are both hydrogen and n is zero or one; and

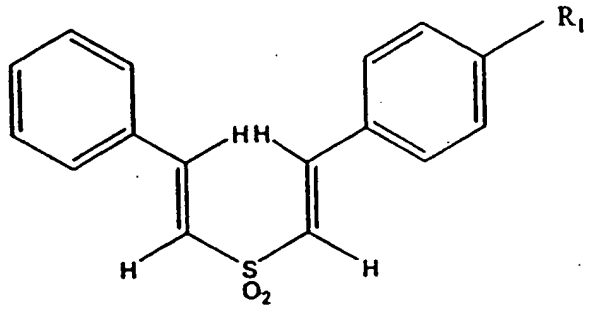
R_1, R_2 and R_3 may not all be hydrogen when n is one.

20. A composition according to claim 12 wherein the compound is E-4-bromostyryl 4-fluorobenzyl sulfone.

21. A composition according to claim 12 wherein the compound is E-4-chlorostyryl 4-bromobenzyl sulfone.

22. A composition according to claim 12 wherein the compound is E-4-bromostyryl 4-chlorobenzyl sulfone.

23. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound of the formula III

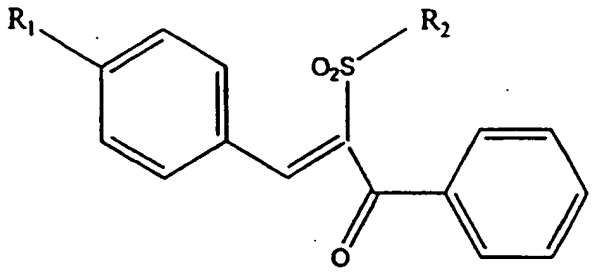


III

wherein

R_1 is selected from the group consisting of hydrogen, chlorine, fluorine and bromine.

24. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the compound of formula IV



IV

wherein

R_1 is selected from the group consisting of fluorine and bromine, and R_2 is selected from the group consisting of 2-chlorophenyl, 4-chlorophenyl, 4-fluorophenyl and 2-nitrophenyl.

25. A method of treating an individual for cancer comprising administering to said individual an effective amount of a pharmaceutical composition according to claim 9.

26. A method of inhibiting growth of tumor cells in an individual afflicted with cancer comprising administering to said individual a pharmaceutical composition according to claim 9.

27. A method of inducing apoptosis of tumor cells in an individual afflicted with cancer comprising administering to said individual a pharmaceutical composition according to claim 9.

28. A method of treating an individual for cancer comprising administering to said individual an effective amount of a pharmaceutical composition according to claim 23 or 24.

29. A method of inhibiting growth of tumor cells in an individual afflicted with cancer comprising administering to said individual a pharmaceutical composition according to claim 23 or 24.

30. A method of inducing apoptosis of tumor cells in an individual afflicted with cancer comprising administering to said individual a pharmaceutical composition according to claim 23 or 24.

31. A method according to claim 25 for treating breast or prostate cancer.

32. A method according to claim 26 for inhibiting growth of breast or prostate tumor cells in an individual afflicted with breast or prostate cancer.

33. A method according to claim 27 for inducing apoptosis of breast or prostate tumor cells in an individual afflicted with breast or prostate cancer.

34. A method according to claim 28 for treating breast or prostate cancer.

35. A method according to claim 29 for inhibiting growth of breast or prostate tumor cells in an individual afflicted with breast or prostate cancer.

36. A method according to claim 30 for inducing apoptosis of breast or prostate tumor cells in an individual afflicted with breast or prostate cancer.

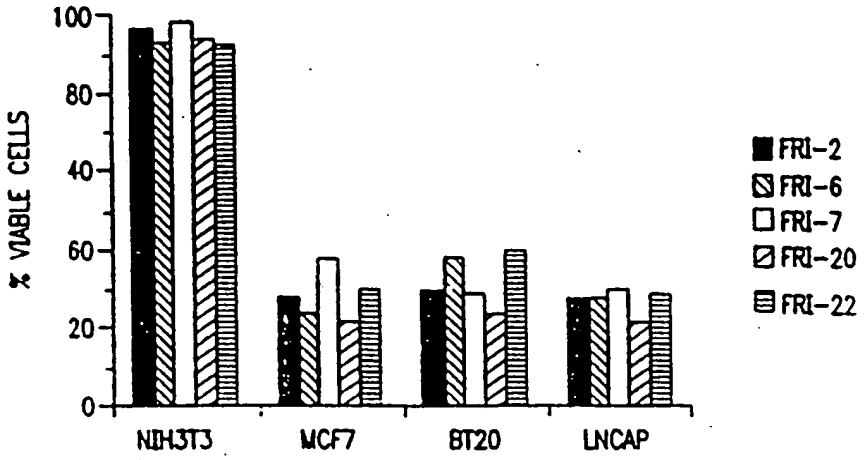

% VIABLE CELLS
100
80
60
40
20
0
NIH3T3
MCF7
BT20
LNCAP
FRI-2
FRI-6
FRI-7
FRI-20
FRI-22

FIG. IA

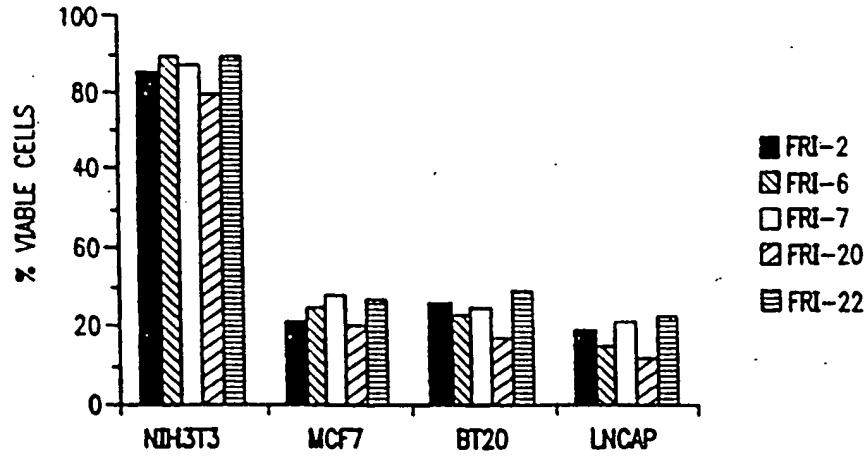

% VIABLE CELLS
100
80
60
40
20
0
NIH3T3
MCF7
BT20
LNCAP
FRI-2
FRI-6
FRI-7
FRI-20
FRI-22

FIG. IB

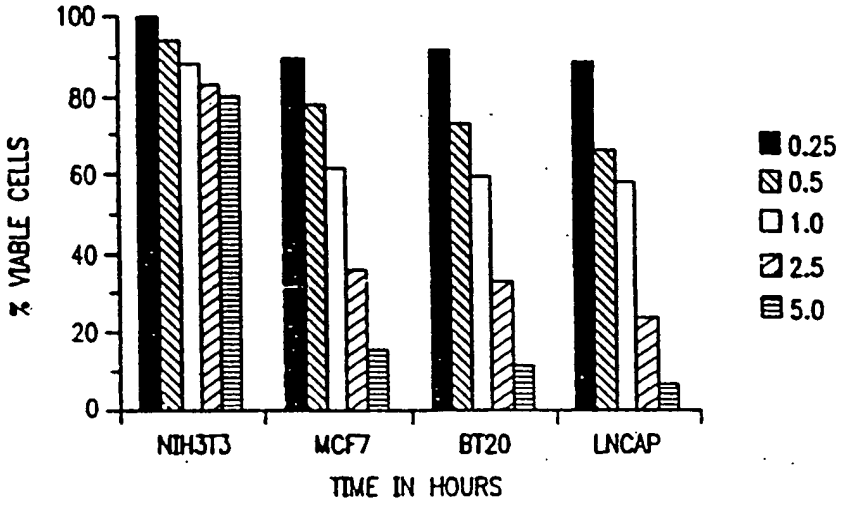

100
80
60
40
20
0
% VIABLE CELLS
NIH3T3
MCF7
BT20
LNCAP
TIME IN HOURS
0.25
0.5
1.0
2.5
5.0

FIG. 2A

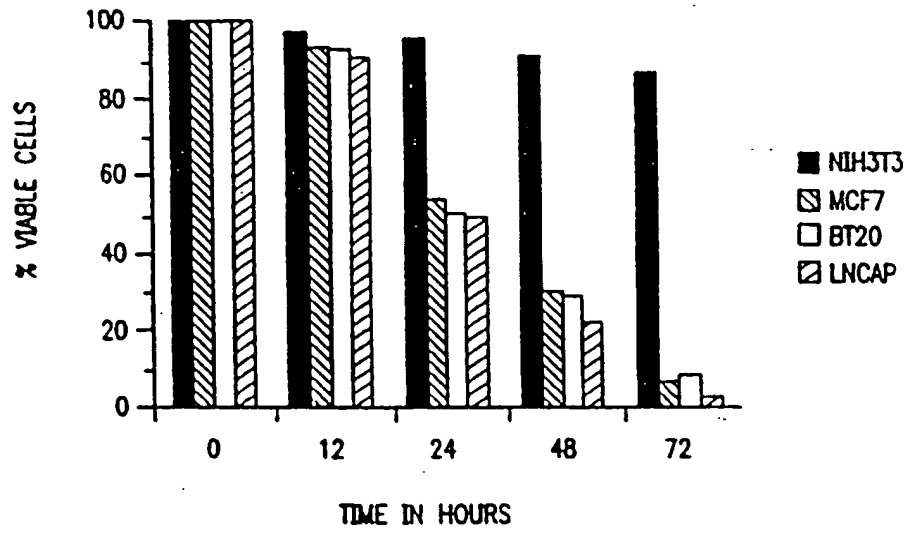

100
80
60
40
20
0
% VIABLE CELLS
0
12
24
48
72
TIME IN HOURS
NIH3T3
MCF7
BT20
LNCAP

FIG. 2B

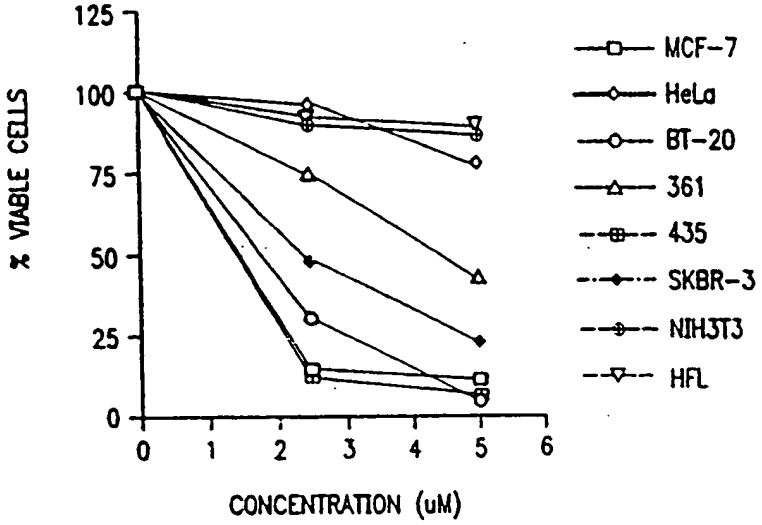

125
100
75
50
25
0
% VIABLE CELLS
0 1 2 3 4 5 6
CONCENTRATION (uM)
MCF-7
HeLa
BT-20
361
435
SKBR-3
NIH3T3
HFL

FIG. 3A

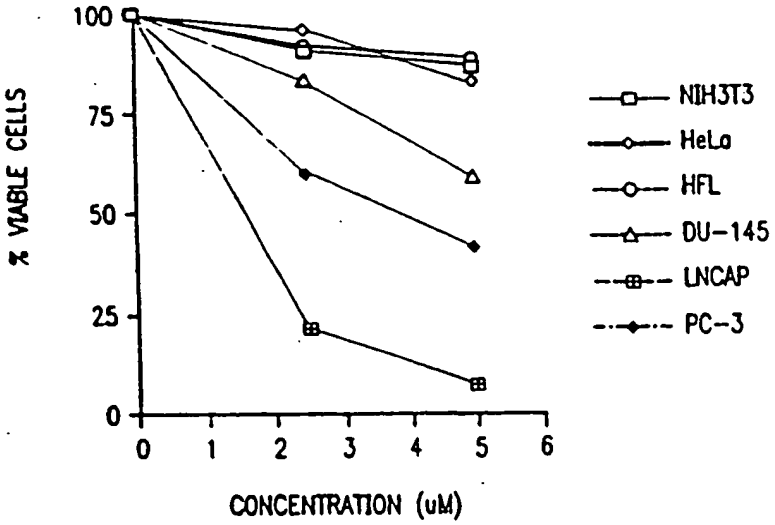

100
75
50
25
0
% VIABLE CELLS
0 1 2 3 4 5 6
CONCENTRATION (uM)
NIH3T3
HeLa
HFL
DU-145
LNCAP
PC-3

FIG. 3B

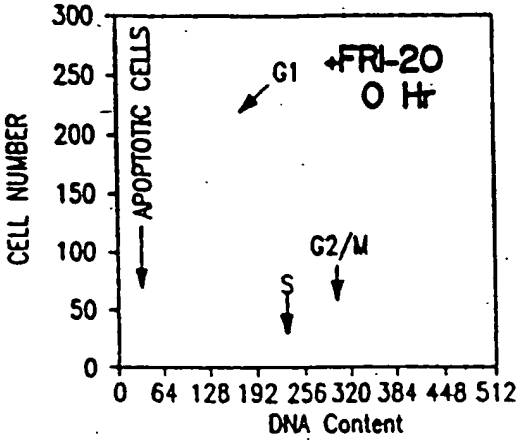


FIG. 4A

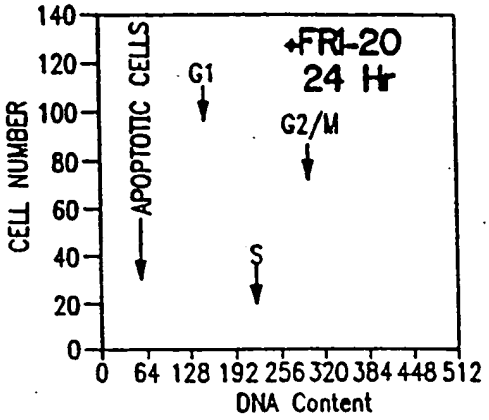


FIG. 4B

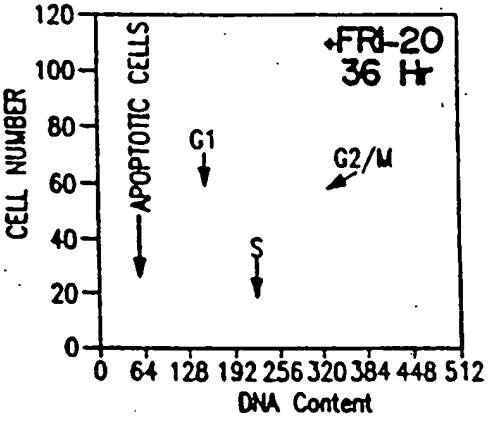


FIG. 4C

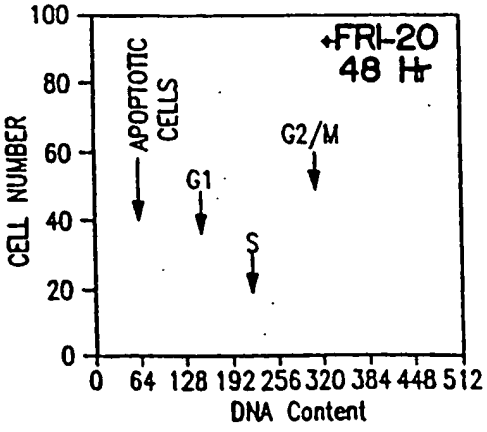


FIG. 4D

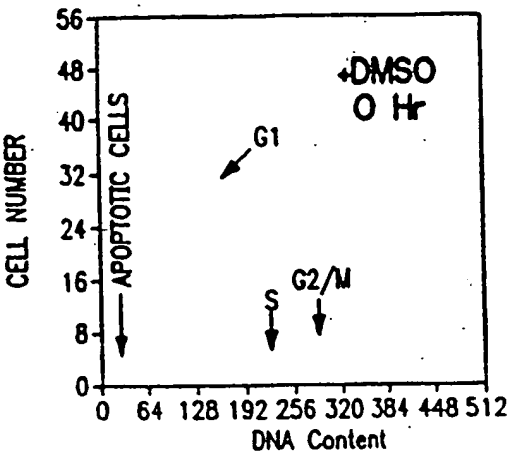


FIG. 4E

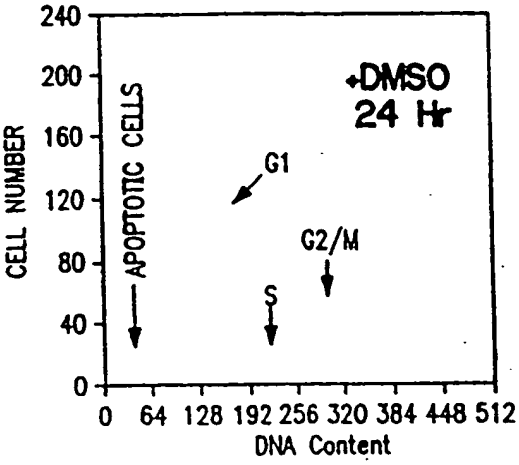


FIG. 4F

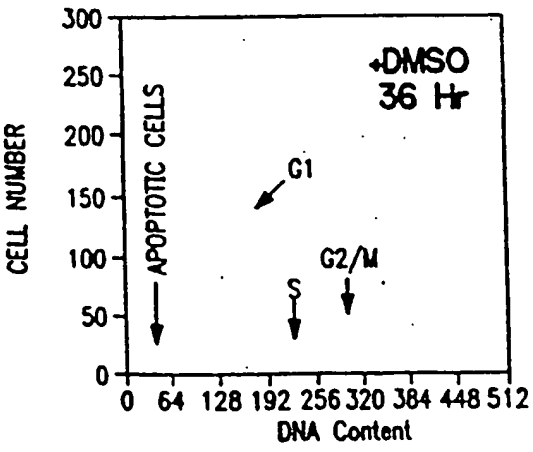


FIG. 4G

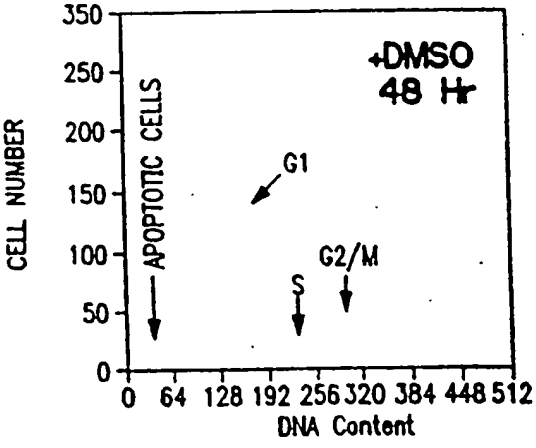


FIG. 4H

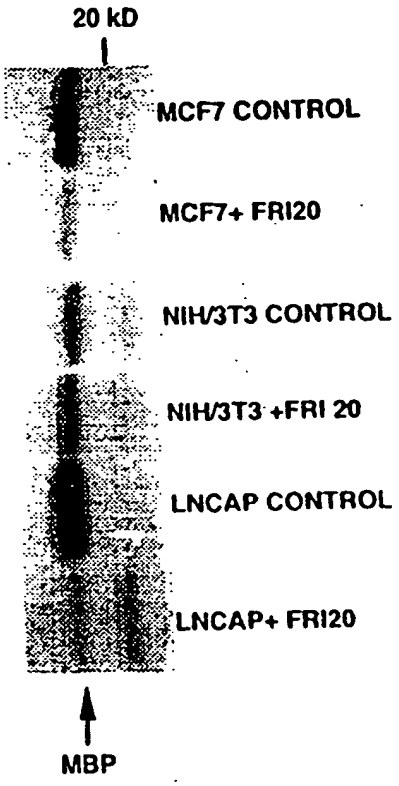

20 kD
MCF7 CONTROL
MCF7+ FRI20
NIH/3T3 CONTROL
NIH/3T3 +FRI 20
LNCAP CONTROL
LNCAP+ FRI20
MBP

FIG. 5

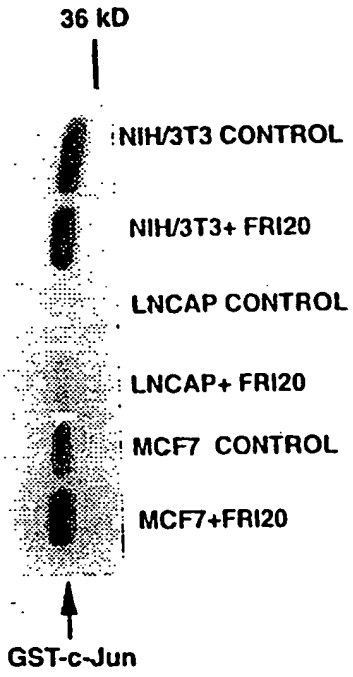

36 kD
NIH/3T3 CONTROL
NIH/3T3+ FRI20
LNCAP CONTROL
LNCAP+ FRI20
MCF7 CONTROL
MCF7+FRI20
GST-c-Jun

FIG. 7

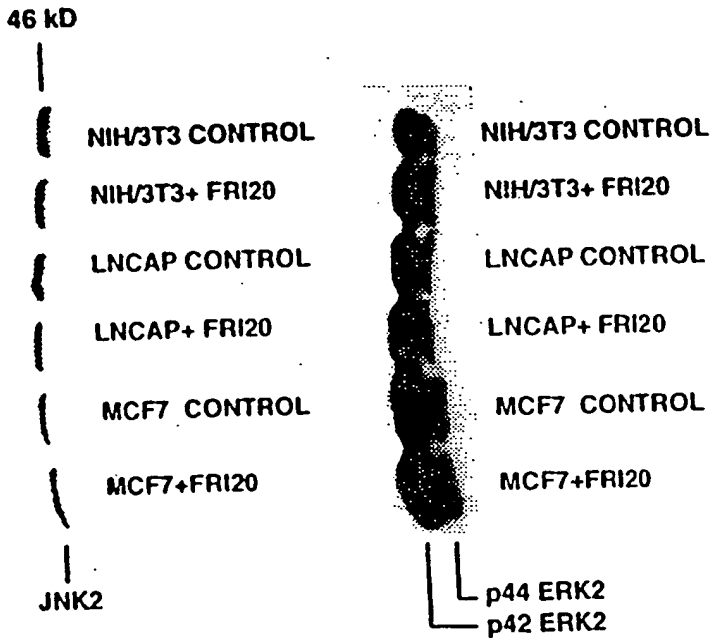

46 kD
NIH/3T3 CONTROL
NIH/3T3+ FRI20
LNCAP CONTROL
LNCAP+ FRI20
MCF7 CONTROL
MCF7+FRI20
JNK2
NIH/3T3 CONTROL
NIH/3T3+ FRI20
LNCAP CONTROL
LNCAP+ FRI20
MCF7 CONTROL
MCF7+FRI20
p44 ERK2
p42 ERK2

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/20580

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07C 317/02, 317/14
US CL : 568/28, 31, 34, 35
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 568/28, 31, 34, 35

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

HAWLEY'S CHEMICAL DICTIONARY , 12th Edition

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Database Caplus on STN, Chem Abstracts, (Columbus OH, USA) No. 124:175763, REDDY, D.B. et al., 'Synthesis of 1,1-disubstitued 2,6-diaryl-4-thian-4,4-dioxides , part-II' abstract Indian J. Heterocycl. Chem., May 1995, Vol. 5, No. 1, pages 11-14.	1-24
A	Database Caplus on STN, Chem Abstracts, (Columbus OH, USA) No. 124:146025, REDDY, D.B. et al., 'E,Z and E,E- bis(styryl) sulfones as precursors of thiane oxides' abstract Indian J. Heterocycl. Chem. April 1995, Vol. 4, No. 4, pages 259-264.	1-24
A	US 4,937,388 A (BUSHELL et al) 26 June 1990 (26.06.90) column 1, lines 20-32 and abstract.	1-22

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 27 NOVEMBER 1998	Date of mailing of the international search report 29 DEC 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer JEAN F. VOLLANO D. Laurence for

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/20580

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	Database Caplus on STN, Chem Abstracts (Columbus OH, USA) No. 126:166162, Thompson, H. et al. 'Sulfone metabolite of sulindac inhibits mammary carcinogenesis' abstract Cancer Res., February 1997, Vol. 57, No. 2, pages 267-271.	25-30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/20580

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAS ONLINE, BEILSTEIN

search terms: breast cancer, prostate cancer, sulfone, malignant tumor, structure drawings

EXHIBIT C

L1 ANSWER 1 OF 1 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
AN **1998287056** EMBASE
TI Reducing the toxicity of anticancer therapy: New strategies.
AU Griggs J.J.
CS J.J. Griggs, University Rochester Cancer Center, 601 Elmood Avenue, Rochester, NY 14642, United States
SO Leukemia Research, (1998) 22/SUPPL. 1 (S27-S33).
Refs: 34
ISSN: 0145-2126 CODEN: LEREDD
PUI S 0145-2126(98)00036-8
CY United Kingdom
DT Journal; Conference Article
FS 016 Cancer
025 Hematology
030 Pharmacology
037 Drug Literature Index
038 Adverse Reactions Titles
052 Toxicology
LA English
SL English

=> d ab

L1 ANSWER 1 OF 1 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
AB Cytoprotective agents offer opportunities to reduce the treatment-related toxicity of anticancer therapy and perhaps to increase the dose and dose intensity of radiation and chemotherapy. One such agent is amifostine, an organic thiophosphate. Amifostine selectively protects normal tissues and provides broad-spectrum protection for a variety of organs while remaining minimally toxic. Clinical studies have demonstrated that amifostine protects against myelotoxicity, nephrotoxicity, neurotoxicity, mucositis and esophagitis in patients treated with alkylating and platinum agents, paclitaxel and radiation therapy. In addition, preclinical studies suggest the possibility of protection against anthracycline induced cardiotoxicity and radiation- and chemotherapy-induced mutagenicity. Preclinical and clinical studies have not demonstrated any diminution of antitumor efficacy. Amifostine is well tolerated in doses of 740 or 910 mg/m2. The most common side effects requiring treatment are transient hypotension, which responds to intravenous fluids, and nausea and vomiting, effectively treated with 5-HT, antagonists and dexamethasone.

EXHIBIT D

THE MERCK INDEX

AN ENCYCLOPEDIA OF
CHEMICALS, DRUGS, AND BIOLOGICALS

TWELFTH EDITION

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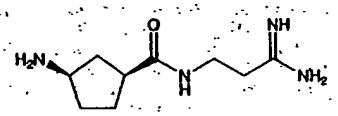
Published by
Merck Research Laboratories
Division of
MERCK & CO., INC.
Whitehouse Station, NJ

1996

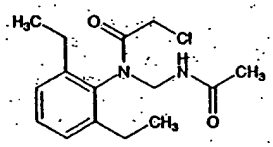
Antibiotic substance produced by *Streptomyces flavochromogenes* isolated from Japanese soil (Shiuoka Prefecture). Isoln and structure: S. Nakamura *et al., J. Antibiot.* 14A, 103 (1961); S. Nakamura, *Chem. Pharm. Bull.* 9, 641 (1961). Identity with myxoviromycin: S. Nakamura *et al., J. Antibiot.* 14A, 163 (1961). Prepn: Katsube, Saito, Japan. pat. 21,418('68) (to Sumitomo), *C.A.* 70, 87135q (1969). Synthesis of amidinomycin and *trans* isomer: H. Paul *et al., Arch. Pharm.* 301, 512 (1968). Crystal and molecular structure: M. Kaneda *et al., J. Antibiot.* 33, 778 (1980).



Sulfate, $C_9H_{18}N_4O.H_2SO_4$, plates or needles from water + methanol, dec 285-288°. $[\alpha]_D^{21}$ −3.9° (c = 3). Absorption spectra: S. Nakamura, *loc. cit.* Soluble in water. Practically insol in ether, benzene, ethyl acetate, methanol, ethanol, butanol, acetone.

THERAP CAT: Antiviral.

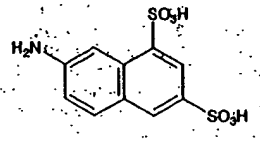
420. Amidochlor. *N-[(Acetylamino)methyl]-2-chloro-N-(2,6-diethylphenyl)acetamide; N*-acetamidomethyl-2-chloro-2',6'-diethylacetanilide; MON-4621; Limit. $C_{15}H_{21}ClN_2O_2$; mol wt 296.80. C 60.70%, H 7.13%, Cl 11.95%, N 9.44%, O 10.78%. Plant growth regulator for cool season grasses. Prepn: Neth. pat. Appl. 7,207,261; K. W. Ratts, U.S. pat. 3,830,841 (1972, 1974 both to Monsanto); K. W. Ratts, J. P. Chupp, *J. Org. Chem.* 39, 3745 (1974). Use as plant growth regulator: K. W. Ratts *et al.*, U.S. pat. 3,829,306 (1974 to Monsanto). Effect on growth and seedhead suppression of annual bluegrass: A. M. Petrovic *et al., Agron. J.* 77, 670 (1985); of wild and cultivated proso millet: J. L. Carpenter, H. J. Hopen, *HortScience* 20, 942 (1985); on established turfgrass: P. C. Bhowmik, *Proc. 5th Int. Turfgrass Res. Conf.* 735 (1985).



Crystals from methanol, mp 148-149°.

USE: Turf growth regulator.

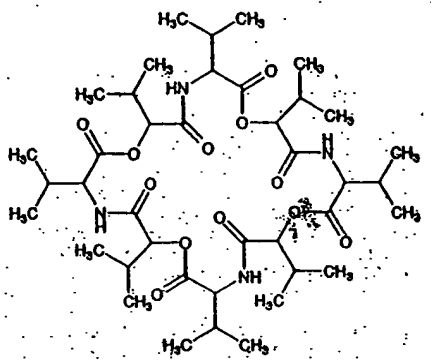
421. Amido-G-Acid. *7-Amino-1,3-naphthalenedisulfonic acid;* 2-naphthylamine-6,8-disulfonic acid; amino-G-acid. $C_{10}H_9NO_6S_2$; mol wt 303.32. C 39.60%, H 2.99%, N 4.62%, O 31.65%, S 21.14%. Prepd by sulfonation of β-naphthylamine: Fierz-David, Braunschweig, *Helv. Chim. Acta* 6, 1146 (1923).



Tetrahydrate, fine monoclinic needles. Sol in water, less sol in alc. Soly in water at 20°: 9.24 g in 100 g of satd soln.

USE: Manufacture of dyes.

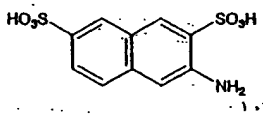
422. Amidomycin. $C_{40}H_{68}N_4O_{12}$; mol wt 797.00. C 60.28%, H 8.60%, N 7.03%, O 24.09%. Antibiotic substance produced by an unidentified *Streptomyces* culture (PRL 1642). Composed of 4 moles each of D-(−)-valine and D-(−)-α-hydroxyisovaleric acid, linked alternately by ester and amide bonds to form a 24-membered ring: Vining, Taber, *Can. J. Chem.* 35, 1109 (1957). Structure studies: Shemyakin *et al., Tetrahedron Letters* 1963, 351, *Tetrahedron* 19, 995 (1963).



D-amino acids only

Needles from dilute ethanol or petr ether, mp 192°. $[\alpha]_D^{25}$ +19.2° (c = 1.2 in ethanol). Neutral reaction. Practically insol in water. Readily sol in most organic solvents. Primarily active against yeasts.

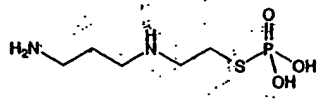
423. Amido-R-Acid. *3-Amino-2,7-naphthalenedisulfonic acid;* 2-naphthylamine-3,6-disulfonic acid. $C_{10}H_9NO_6S_2$; mol wt 303.32. C 39.60%, H 2.99%, N 4.62%, O 31.65%, S 21.14%. Prepd by treating 2-hydroxy-3,6-naphthalenedisulfonic acid with ammonium sulfite and ammonium hydroxide: Petitcolas, Josué, *Bull. Soc. Chim. France* 1952, 89.



Crystals or powder. Soluble in water. Solutions show a violet-blue fluorescence.

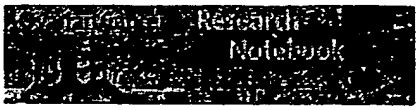
USE: Manufacture of dyes.

424. Amifostine. *2-[(3-Aminopropyl)amino]ethanethiol dihydrogen phosphate ester; phosphorothioic acid S-[2-[(3-aminopropyl)amino]ethyl] ester;* aminopropylaminoethyl thiophosphate; ethiofos; gammaphos; SAPEP; NSC-296961; WR-2721; YM-08310; Ethyol. $C_5H_{15}N_2O_3PS$; mol wt 214.23. C 28.03%, H 7.06%, N 13.08%, O 22.41%, P 14.46%, S 14.97%. Thiophosphate derivative of cysteamine, *q.v.*; provides normal cells with selective protection against the toxic effects of cancer chemotherapy and radiation treatment. Prepn of monohydrate: J. R. Piper *et al., J. Med. Chem.* 12, 236 (1969); J. R. Piper, T. P. Johnston, U.S. pat. 3,892,824 (1975 to Southern Res. Inst.). Differential radioprotective activity: J. M. Yuhas, J. B. Storer, *J. Nat. Cancer Inst.* 42, 331 (1969). Mechanism of action study: G. D. Smoluk *et al., Cancer Res* 48, 3641 (1988). Bioavailability: L. Fleckenstein *et al., Pharmacol. Ther.* 39, 203 (1988). Clinical pharmacokinetics: L. M. Shaw *et al., ibid.* 195. HPLC determn in plasma: N. F. Swynnerton *et al., Int. J. Radiat. Oncol. Biol. Phys.* 12, 1495 (1986). Review of development as radioprotector: D. Q. Brown *et al., Pharmacol. Ther.* 39, 157-168 (1988); of role in chemotherapy: R. L. Capizzi *et al., Cancer* 72, 3495-3501 (1993); M. Treskes, W. J. M. van der Vijgh, *Cancer Chemother. Pharmacol.* 33, 93-106 (1993).



Monohydrate, white solid from methanol/ether, mp 160-161° (dec). LD_{50} in mice (mg/kg): 700 i.p. (Piper, Johnston).

EXHIBIT E



Cytoprotectant Amifostine Approved

The FDA has approved a new cytoprotective agent -- amifostine (Ethyol/US Bioscience) -- for reducing the cumulative renal toxicity associated with repeated cisplatin therapy in patients with advanced ovarian cancer. Amifostine is an organic thiophosphate prodrug that is rapidly dephosphorylated in tissues to a pharmacologically-active free thiol. This thiol compound binds and detoxifies reactive cisplatin metabolites, and also scavenges free radicals generated in tissues exposed to cisplatin. Since amifostine reaches a higher concentration in normal tissue relative to tumor tissue, it is able reduce cisplatin's renal toxicity without compromising the antitumor efficacy. First developed to protect tissues against radiation damage, amifostine has since proved to be effective for protecting against hematologic toxicity in patients receiving cisplatin, cyclophosphamide, and/or mitomycin, and to reduce cisplatin- induced nephrotoxicity, ototoxicity, and neurotoxicity. In clinical trials, patients treated with amifostine showed a reduction in neutropenia-related fever and sepsis and spent fewer days in the hospital and/or on antibacterial therapy, compared with patients who did not receive amifostine. Moreover, fewer patients discontinued therapy before completing the scheduled number of treatment cycles. Amifostine is given intravenously as a 15-minute infusion of 910 mg/m2 starting 30 minutes prior to cisplatin therapy.

The drug is rapidly cleared from plasma; distribution half-life is less than one minute and elimination half-life is about 8 minutes. Less than 10% of amifostine remains in the plasma 6 minutes after administration. The most common side effects are transient reduction in blood pressure, nausea, vomiting, somnolence, and sneezing. Less common side effects include flushing, hypocalcemia, and hiccups. Antiemetics reduce the nausea (pretreatment with dexamethasone or metoclopramide has no effect on pharmacokinetics). Contraindications include hypotension, dehydration, hypocalcemia, or sensitivity to aminothiol compounds or mannitol (the vehicle). US Bioscience is continuing Phase II and III trials to evaluate the effects of ethyol for protecting against radiation damage in patients treated for tumors of the rectum, cervix, lung and neck. It is also under investigation for its ability to sensitize a tumor to therapy. It is in trial for use with Bristol-Myer's taxol, allowing escalation of the dose to more than twice the dosage currently used in clinical practice. US Bioscience developed amifostine and owns the patents; Alza has exclusive marketing rights in the US for five years; Schering-Plough is marketing the drug in Europe; and Lilly is planning to introduce it in Canada. (Spencer CM, Goa KL. Drugs 1995;50:1001-1031. Additional information from Alza.)

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